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Antagonist resistant contractions of the porcine pulmonary artery by cysteinyl-leukotrienes

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Abstract

The contractile response to cysteinyl-leukotrienes was studied in isolated porcine pulmonary arterial rings. In endothelium-denuded preparations, the concentration–response curves for leukotriene C_4 and leukotriene D_4 were identical, whereas leukotriene E_4 did not contract these tissues. The response to leukotriene C_4 was not blocked by either $CysLT_1$/$CysLT_2$ receptor antagonism or by pre-treatment with leukotriene E_4. In preparations with an intact endothelium, leukotriene C_4 was somewhat more potent than leukotriene D_4 and the concentration–response curves were only slightly depressed in the presence of either ICI 204,219 (4-(5-cyclopentyloxycarbonylamino-1-methylindol-3-ylmethyl)-3-methoxy-*N*-*o*-tolylsulfonylbenzamide, 1 μM) or BAY u9773 (6(*R*)-(4′-carboxyphenylthio)-5(*S*)-hydroxy-7(*E*),9(*E*),11(*Z*)14(*Z*)-eicosatetrenoic acid, 3 μM). Indomethacin (1.7 μM) significantly reduced the response to leukotriene C_4 whereas the response to leukotriene D_4 was unchanged. These findings suggest that a CysLT receptor subtype resistant to current antagonists mediated the major part of the contractions to leukotriene C_4 and leukotriene D_4 in intact preparations, and was the sole receptor associated with contractions of endothelium-denuded preparations.

Keywords: Pulmonary artery, porcine; Leukotriene; Contraction; CysLT receptor; ICI 204,219; BAY u9773

1. Introduction

In isolated human airways, all cysteinyl-leukotrienes (leukotriene C_4, D_4 and E_4) are equipotent and activate a single receptor leading to contraction (Buckner et al., 1986). These contractions are blocked by the class of antagonists developed for treatment of asthma, and the receptor, referred to as $CysLT_1$ (Coleman et al., 1995), has recently been cloned (Lynch et al., 1999; Sarau et al., 1999).

Previous reports (Drazen et al., 1980; Fleisch et al., 1982; Snyder and Krell, 1984) had however demonstrated that in the guinea pig, smooth muscle contractions induced by leukotriene C_4 were due to activation of a receptor, which was different from that stimulated with leukotriene D_4 and leukotriene E_4. All selective $CysLT_1$ receptor antagonists (Krell, 1989) block specifically the leukotriene D_4- or E_4-but not the leukotriene C_4-induced contractions in the guinea pig. In 1992, Labat et al. (1992) demonstrated the presence of this second CysLT receptor in the human lung, where it is associated with contraction of pulmonary venous vascular smooth muscle and activated by all cysteinyl-leukotrienes. This receptor was resistant to $CysLT_1$ receptor antagonists, but blocked by the leukotriene E_4 analogue BAY u9773, and is referred to as $CysLT_2$ (Coleman et al., 1995). Ortiz et al. (1995) extended these observations by demonstrating the presence of CysLT receptors at the level of the endothelium in the human pulmonary venous vascular bed. Together, these observations suggested that two receptors ($CysLT_1$ and $CysLT_2$ receptors) were present in the human pulmonary vasculature. The existence of several subtypes of receptors for cysteinyl-leukotrienes has also received support from radioligand binding studies on human lung parenchyma membranes using [^{3}H]-labelled leukotrienes as agonists (Rovati et al., 1985, Capra et al., 1998).

In vivo studies have shown that the cysteinyl-leukotrienes produce marked alterations in pulmonary arterial pressure when injected into different species (Smedegård

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et al., 1982; Berkowitz et al., 1984; Kadowitz and Hyman, 1984). Ohtaka et al. (1987) showed that in the pig, leukotriene C_4 markedly increased the pulmonary arterial pressure as well as pulmonary capillary wedge pressure, an effect, which was less pronounced when leukotriene D_4 was injected. These investigators also suggested that some effects of leukotriene C_4 were due to the generation of thromboxane A_2. However, these and other (Leffler et al., 1984; Olson and Fleisher, 1989; Zellner et al., 1991) in vivo studies of cysteinyl-leukotrienes and $CysLT_1$ receptor antagonists in the pig have not established, which CysLT receptors are involved. In addition, results from in vivo studies are difficult to interpret since direct effects on the pulmonary vessels may be masked by cardiovascular reflexes. Isolated tissues offer an appropriate model for identification and characterisation of the CysLT receptors specifically at the pulmonary vascular level.

To date, there have been no systematic studies performed to characterise the CysLT receptors in the porcine vascular bed. Therefore, the aim of this study was to determine the CysLT receptor subtype mediating functional responses in porcine pulmonary arteries, using isolated pulmonary arterial ring preparations.

2. Materials and methods

2.1. Tissue preparation

Fourteen male pigs (large white), 3–4 months of age, weighing 22 ± 1.4 kg (mean $\pm$ S.E.M.) were premedicated with atropine (0.5 mg i.m.) and then anaesthetised with ketamine (100 mg/kg i.m.) followed by pentobarbital sodium (25 mg/kg i.v.). Heparine (3 mg/kg) was also administered. Subsequent to thoracotomy, the animals were exsanguinated and the lungs were removed and intrapulmonary arteries, representing the first branches of the main lobar artery, 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 2 g and were left to equilibrate in the organ baths for 90 min with changes of Tyrode's solution every 10 min. Changes in isometric tension were recorded via Narco F-60 force-displacement transducers connected to a Linseis 2016 polygraph. The experimental protocol started with the administration of a single dose of noradrenaline (10 μM) in order to test tissue viability and to obtain a reference contraction. This contraction was: 2.26 ± 0.22 g ($n = 12$ pigs with an average of nine preparations/pig), which was not significantly different from that obtained in rubbed preparations (2.88 ± 0.39 g, $n = 12$ pigs, five preparations/pig). After reequilibration with washes every 10 min, the preparations were incubated for 30 min in the absence (control) or presence of ICI 204,219 (1 μM, $CysLT_1$ receptor antagonist), BAY u9773 (3 and 10 μM, $CysLT_1$ and $CysLT_2$ receptor antagonist), indomethacin (1.7 μM, cyclooxygenase inhibitor) or leukotriene E_4 (1 μM). At the end of the 30-min treatment, cumulative concentration–response curves for either leukotriene C_4 or leukotriene D_4 were established by the addition of increasing concentrations (0.1 nM–1 μM). Higher concentrations of the cysteinyl-leukotrienes were not used due to the costs involved. When the cysteinyl-leukotriene contraction reached a plateau at the end of the cumulative dosing, noradrenaline (10 μM) was administered to the preparations, followed by acetylcholine (10 μM). There was no significant difference in this noradrenaline contraction between controls and the different treatments. 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. In order to explore the cysLT receptor associated with contraction of the vascular smooth muscle, concentration–response curves for leukotriene E_4 were established only in rubbed preparations (eight preparations from two pigs).

In preliminary experiments, the effect of inhibition of the metabolism of cysteinyl-leukotrienes was examined by treating the tissues with a γ-glutamyltranspeptidase inhibitor (L-serine borate, 45 mM) and a dipeptidase inhibitor (L-cysteine, 5 mM) before generation of concentration–effect curves to either leukotriene C_4 or leukotriene D_4, respectively. These enzyme inhibitors did not cause any apparent change in the concentration–effect relations for the cysteinyl-leukotrienes, and subsequent experiments were performed in the absence of these inhibitors.

2.3. Mediator release measurements

Four 500-μl aliquots were taken directly from the organ bath and immediately frozen at −20°C. The first sample was taken after a 10-min period at the end of the equilibration period, the second at the plateau of the first maximal contraction to noradrenaline (10 min, 100% contraction). The third sample after a 10-min period prior to drug treatment and the fourth at 10 min after the administration of the last and highest concentration of leukotriene C_4 and D_4, representing a contraction of about 70% and 50% of noradrenaline, respectively (see Results). The first and

third samples represent the basal levels and were subtracted from the levels obtained after noradrenaline (sample 2) and cysteinyl-leukotrienes (sample 4), respectively.

Direct quantification of the stable metabolites thromboxane B_2 and 6-keto prostaglandin $F_{1\alpha}$ was performed by enzyme immunoassay (Stallergenes, Fresnes, France) as previously described in detail by Pradelles et al. (1985). Since repeated measurements of thromboxane B_2 in organ bath fluid samples from single preparations were below the detection limit, the bath fluid from three to four different preparations were pooled together and evaporated in order to obtain detectable levels of thromboxane B_2.

2.4. Drugs

Noradrenaline, acetylcholine, indomethacin, L-cysteine, L-serine and boric acid were obtained from Sigma (St. Louis, MO, USA). Leukotrienes C_4, D_4 and E_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) was a gift from BAYER (Stokes Poges, Great Britain). ICI 204,219 (4-(5-cyclopentyloxycarbonylamino-1-methylindol-3-ylmethyl)-3-methoxy-*N*-*o*-tolylsulfonylbenzamide) was from Zeneca (Wilmington, DE, USA).

Solutions of cysteinyl-leukotrienes and BAY u9773 were obtained by diluting stock solutions at concentrations of 1 mM (leukotriene C_4 and leukotriene E_4), 0.5 mM (leukotriene D_4) and 10 mM (BAY u9773) into Tyrode's solution. Indomethacin was dissolved in 1% ethanol in Tyrode's solution, ICI 204,219 in dimethyldisulfoxide (the final bath concentration of the solvents being less than 0.1%). L-serine borate was prepared from 1-M concentrations of L-serine and boric acid dissolved in water and buffered at pH 7.4 with 10 M NaOH. Noradrenaline, acetylcholine and L-cysteine were dissolved in Tyrode's solution.

2.5. Data analysis

All data are expressed as means ± S.E.M. The contractions are expressed as increased tension in grams or as percent of the initial noradrenaline response. The results of the mediator measurements were expressed as released substance in pg mg^{-1} of tissue wet weight. The increase in release was calculated by subtracting the basal level of each sample from the value obtained after stimulation. Statistical evaluation was performed using a two-way analysis of variances (ANOVA) test (concentration–response curves) or a Student's *t*-test. A *P*-value of less than 0.05 was considered significant.

3. Results

3.1. Contractions

Leukotriene C_4 and leukotriene D_4 contracted both intact and rubbed rings from porcine pulmonary arteries in a concentration dependent manner. The contractions induced by leukotriene C_4 (1 μM) were 1.8 ± 0.4 g ($n = 6$) in intact and 1.3 ± 0.2 g ($n = 12$) in rubbed preparations.

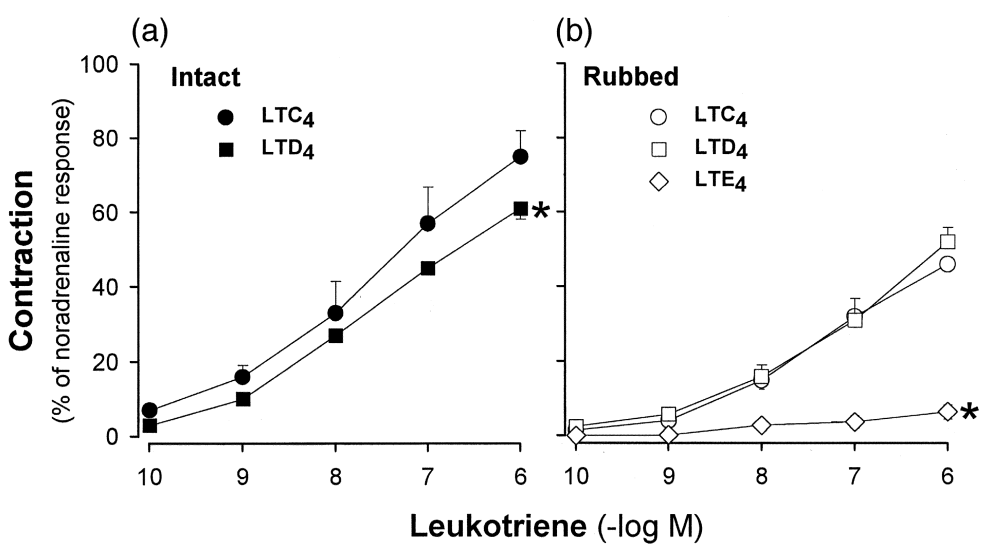

Fig. 1. Concentration–effect relations for (a) leukotriene C_4 (LTC_4) and leukotriene D_4 (LTD_4) in intact and for (b) leukotriene C_4, D_4 and E_4 (LTC_4, LTD_4 and LTE_4,) in rubbed isolated porcine pulmonary arterial ring preparations. The contractions are presented as percent of an initial contraction to noradrenaline (10 μM) and vertical lines represent S.E.M. In (a), each point is the mean of preparations obtained from four different pigs (paired observations) and * indicates a significant difference ($P < 0.05$, two-way ANOVA test) between the two curves. In (b), each point is the mean of: 12 preparations from 10 different pigs (leukotriene C_4), six preparations from five pigs (leukotriene D_4) and eight preparations from two pigs (leukotriene E_4). * indicates that the leukotriene E_4 curve was significantly different ($P < 0.05$, two-way ANOVA test) from leukotriene C_4 and leukotriene D_4 curves.

For leukotriene D_4 (1 μM), the contractions were 1.2 ± 0.2 g ($n = 6$) in intact and 1.3 ± 0.3 g ($n = 7$) in rubbed preparations. The contractions to leukotriene E_4 (1 μM) were 0.17 ± 0.06 g (eight preparations from two pigs) in rubbed preparations. In intact preparations, there was a small but significant different effect between the concentration–response curves for leukotriene C_4 and those established for leukotriene D_4 (Fig. 1a). In endothelium-denuded preparations, the concentration–response curves for leukotrienes C_4 and leukotriene D_4 were identical. In contrast, contractions to leukotriene E_4 were negligible (Fig. 1b).

ICI 204,219 ($CysLT_1$ receptor antagonist) and BAY u9773 ($CysLT_1$ and $CysLT_2$ receptor antagonist) caused small but significant inhibition of the concentration–response curves to leukotriene C_4 and leukotriene D_4 in preparations with an intact endothelium (Fig. 2). Since the effect of the antagonists was most pronounced against leukotriene C_4, this agonist was further studied in rubbed preparations. The contractions induced by leukotriene C_4 in the rubbed preparations were not inhibited by BAY u9773 (3 μM) or leukotriene E_4 (1 μM, Fig. 3). Neither did pretreatment of rubbed preparations with leukotriene E_4 (1 μM) seem to modify the contractions to leukotriene D_4 ($n = 1$). A higher dose of BAY u9773 (10 μM) was also unable to inhibit the leukotriene C_4 contractions ($n = 1$).

In preparations with an intact endothelium indomethacin treatment did not alter the second noradrenaline response (Controls: 2.8 ± 0.52 g and indomethacin: 2.6 ± 0.52 g, $n = 4$ paired data), but significantly reduced the response to leukotriene C_4 without changing the concentration–response curve for leukotriene D_4 (Fig. 4).

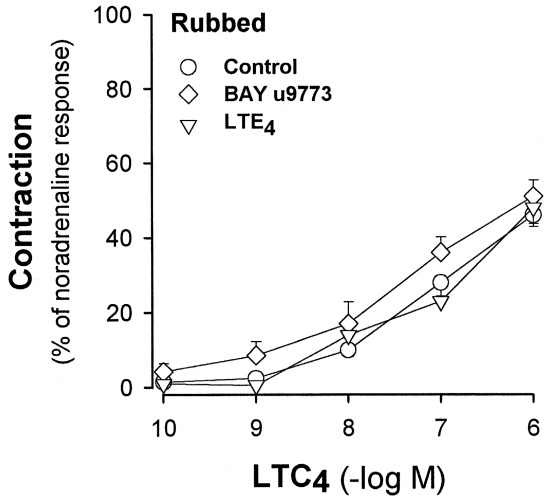

Fig. 3. Effect of BAY u9773 (3 μM, $CysLT_1$ /$CysLT_2$ receptor antagonist) and leukotriene E_4 (1 μM) on the concentration–effect relation for leukotriene C_4 in isolated porcine pulmonary arterial ring preparations where the endothelium had been mechanically removed (rubbed). Contractions are presented as percent of an initial contraction to noradrenaline (10 μM) and vertical lines represent S.E.M. Each point is the mean of preparations derived from six (control), five (BAY u9773) or two to three (leukotriene E_4) different pigs.

3.2. Mediator release measurements

Leukotriene C_4 and leukotriene D_4 (1 μM) increased the release of thromboxane A_2 (determined by measurements of thromboxane B_2) by 3.0 and 1.9 pg mg^{-1} tissue wet weight, respectively. The increase in thromboxane B_2 release caused by noradrenaline (10 μM) was similar to

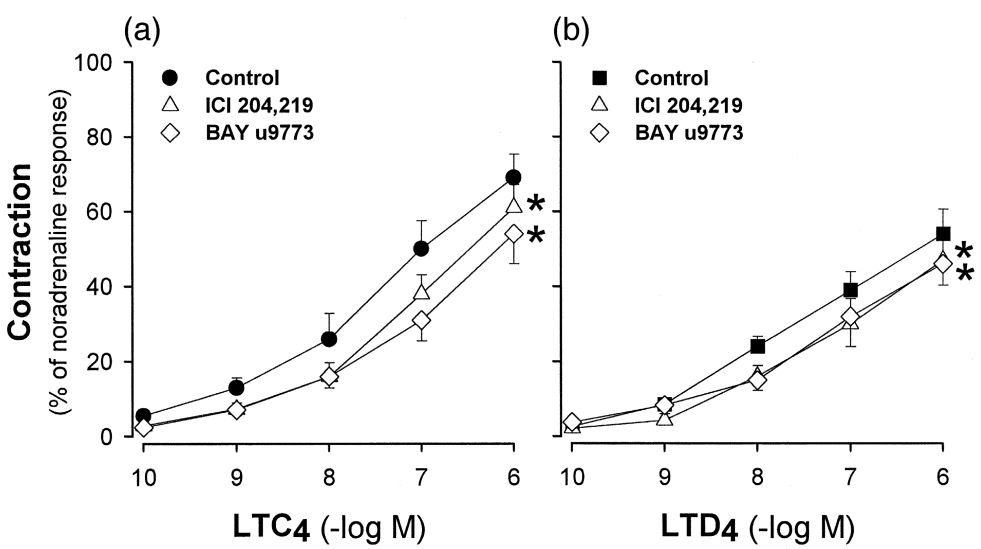

Fig. 2. Effect of ICI 204,219 (1 μM, $CysLT_1$ receptor antagonist) and BAY u9773 (3 μM, $CysLT_1$/$CysLT_2$ receptor antagonist) on the concentration–response curves for (a) leukotriene C_4 (LTC_4) and (b) leukotriene D_4 (LTD_4) in intact isolated porcine pulmonary arterial ring preparations. Contractions are presented as percent of an initial contraction to noradrenaline (10 μM). Each point is the mean of preparations derived from six (a) to five (b) different pigs (paired observations) and vertical lines represent S.E.M. * indicates that the curves were significantly different ($P < 0.05$, two-way ANOVA test) from control curves.

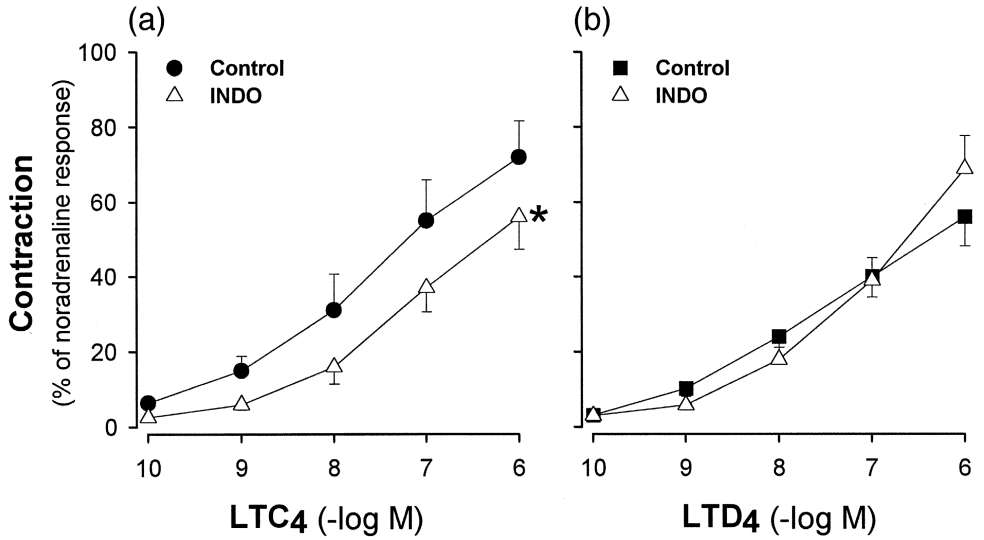

Fig. 4. Effect of indomethacin (INDO, 1.7 μM, cyclooxygenase inhibitor) on the concentration–response curves for (a) leukotriene C_4 (LTC_4) and (b) leukotriene D_4 (LTD_4) in intact isolated porcine pulmonary arterial ring preparations. Contractions are presented as percent of an initial contraction to noradrenaline (10 μM). Each point is the mean of preparations derived from five (a) to four (b) different pigs (paired observations) and vertical lines represent S.E.M. * indicates a significant difference ($P < 0.05$, two-way ANOVA test) from leukotriene C_4 control curve.

that caused by the cysteinyl-leukotrienes: 6.6 pg mg^{-1} tissue. The increase in thromboxane B_2 after either leukotriene C_4 or leukotriene D_4 was not prevented by ICI 204,219 (1 μM; 5.0 and 4.4 pg mg^{-1} tissue, respectively) or BAY u9773 (3 μM; 5.2 and 3.5 pg mg^{-1} tissue, respectively). In contrast, after treatment with indomethacin, the levels of thromboxane B_2 detected subsequent to cysteinyl-leukotriene stimulation were below basal release levels. In rubbed preparations, the release of thromboxane B_2 was 1.3 pg mg^{-1} tissue for leukotriene C_4 and 1.8 pg mg^{-1} tissue for leukotriene D_4.

The basal levels of 6-keto prostaglandin $F_{1\alpha}$ were 107 ± 20 pg mg^{-1} tissue (24 samples from four different pigs). There was a significant increase (approximately seven- to nine-fold) after stimulation with either leukotriene C_4 or leukotriene D_4 (Fig. 5). Noradrenaline (10 μM) also

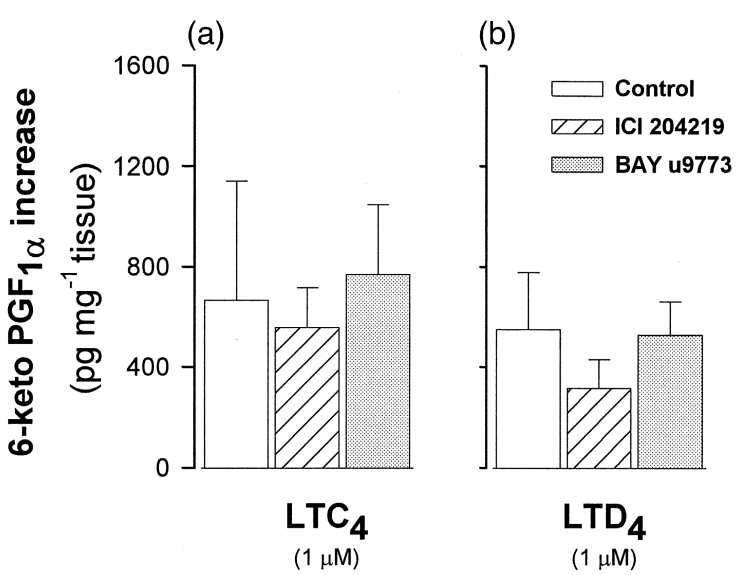

Fig. 5. The release of 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) after stimulation with (a) leukotriene C_4 (LTC_4) and (b) leukotriene D_4 (LTD_4). The increase in release was expressed as pg mg^{-1} tissue wet weight and obtained by subtracting the basal levels (see Results) from the values after stimulation. Each bar is the mean of three measurements of samples derived from three to four different pigs and vertical lines represent S.E.M. ICI 204,219 (1 μM, $CysLT_1$ receptor antagonist) or BAY u9773 (3 μM, $CysLT_1/CysLT_2$ receptor antagonist) did not prevent the increased levels.

increased the 6-keto prostaglandin $F_{1\alpha}$ release although this increase was significantly less than after cysteinyl-leukotrienes (basal: 45 ± 35; noradrenaline, 10 μM: 169 ± 62 pg mg^{-1} tissue, $n = 4$ paired data). The CysLT receptor antagonists did not prevent the increased release of 6-keto prostaglandin $F_{1\alpha}$, which were induced by either leukotriene C_4 or leukotriene D_4 (Fig. 5). In preparations treated with indomethacin, the levels of 6-keto prostaglandin $F_{1\alpha}$ detected during cysteinyl-leukotriene stimulation were not significantly different from basal values (not shown).

4. Discussion

The finding that leukotriene C_4 and leukotriene D_4 contracted the isolated porcine pulmonary arteries are consistent with earlier reports (Ohtaka et al., 1987; Paterson et al., 1988) and show similarities with results obtained in isolated human pulmonary arteries (Schellenberg and Foster, 1984; Bourdillat et al., 1987; Bäck et al., 2000). Ohtaka et al. (1987) previously reported a greater response induced by leukotriene C_4 when compared with leukotriene D_4 using endothelium intact spiral strips of porcine pulmonary artery. The present report, using ring preparations, confirms that study and extends the observation by showing that in rubbed preparations, the concentration–response curves for leukotriene C_4 and leukotriene D_4 were identical whereas the responses to leukotriene E_4 were negligible. These latter observations further support the similarity between porcine and human pulmonary arteries since Schellenberg and Foster (1984) previously reported that leukotriene C_4 and leukotriene D_4 contracted isolated human pulmonary arteries whereas leukotriene E_4 was very weak or inactive.

There are no previous reports concerning the inhibitory effects of CysLT receptor antagonists in porcine pulmonary arterial preparations. The present report demonstrate that in endothelium intact tissues both the selective $CysLT_1$ receptor antagonist ICI 204,219 (1 μM) and the dual $CysLT_1$ and $CysLT_2$ receptor antagonist BAY u9773 (3 μM) only slightly modified the contractions induced by leukotriene C_4 and leukotriene D_4. These effects were observed at a concentration of ICI 204,219 that nearly abolished the responses to leukotriene C_4 and leukotriene D_4 in tissues containing $CysLT_1$ receptors (Buckner et al., 1990; Krell et al., 1990). Likewise, the concentration of BAY u9773 used has previously been found to effectively inhibit $CysLT_1$ and $CysLT_2$ receptor responses (Labat et al., 1992; Tudhope et al., 1994; Bäck et al., 1996; Wikström Jonsson, 1997). The relatively marginal antagonism by ICI 204,219 and BAY u9773 in the present study therefore suggests that there may be another CysLT receptor subtype present in the porcine pulmonary arteries. This is in line with previous studies in the guinea pig lung parenchymal strip where a substantial residual contraction also has been observed after $CysLT_1/CysLT_2$ receptor antagonism (Tudhope et al., 1994; Wikström Jonsson et al., 1998). However, in the lung parenchymal strip, several different target tissues contribute to the contractile response (Brink et al., 1981) and the exact distribution of CysLT receptors is unknown.

Furthermore, although the dual $CysLT_1$ and $CysLT_2$ receptor antagonist, BAY u9773 produced a small but significant depression of the concentration–response curve for leukotriene C_4 in intact preparations, the same treatment did not at all alter the response to leukotriene C_4 in rubbed porcine pulmonary arteries. This latter observation suggests that the CysLT receptor subtype resistant to the antagonists is located at the level of the smooth muscle. These observations provide evidence that the contractile responses to cysteinyl-leukotrienes may be mediated by a receptor resistant to $CysLT_1/CysLT_2$ receptor antagonism. In addition, pretreatment of rubbed preparations with leukotriene E_4 did not modify the contractions to either leukotriene C_4 or D_4. In the isolated human pulmonary veins, this pretreatment has been reported to significantly alter cysteinyl-leukotriene contractions (Labat et al., 1992).

The observation (present report) that the contractions to cysteinyl-leukotrienes were slightly modified by the antagonists in intact, but not in rubbed preparations also suggests the presence of CysLT receptors on the endothelium. While the CysLT receptor on the vascular smooth muscle was resistant to the known antagonists, the receptors on the endothelium appear to be blocked by the $CysLT_1$ receptor antagonist ICI 204,219 as well as by the $CysLT_1/CysLT_2$ receptor antagonist BAY u9773. While the major part of the contractions to cysteinyl-leukotrienes were the result of stimulation of the vascular muscle, endothelial CysLT receptors modulated this response, probably by causing release of endogenous contractile factors. Similar findings have previously been described in human pulmonary vessels (Ortiz et al., 1995).

Ohtaka et al. (1987) showed that indomethacin significantly reduced the contractions to leukotriene C_4 in isolated porcine pulmonary arteries. The present report confirms that study and extends the observation by showing that the contractions to leukotriene D_4 were not altered after treatment with indomethacin. In the study by Ohtaka et al. (1987), the thromboxane synthesis inhibitor, dazoxiben, was shown to cause a somewhat greater inhibition of the leukotriene C_4 response in isolated porcine pulmonary arteries when compared with results obtained with indomethacin. Based on this observation, these investigators suggested that thromboxane A_2 may be the contractile factor, which is released during challenge with leukotriene C_4. However, these investigators did not measure thromboxane release from the porcine pulmonary arterial preparation. The measurements of thromboxane B_2 (present report) showed that the increase in thromboxane B_2 levels after cysteinyl-leukotriene stimulation was very small, about 100 times less than the quantities of 6-keto prosta-

glandin $F_{1\alpha}$ released. In addition, all agonists examined (leukotriene C_4, leukotriene D_4 and noradrenaline) produced the same degree of thromboxane B_2 release but only the leukotriene C_4 contractions were altered by indomethacin treatment. Together, these observations indicate that, in contrast to what has previously been suggested, thromboxane A_2 may not be responsible for the modifications of leukotriene C_4 contractions by indomethacin.

The measurements of 6-keto prostaglandin $F_{1\alpha}$ (Fig. 5) demonstrated that also prostacyclin was released in response to both leukotriene C_4 and leukotriene D_4. Even though prostacyclin is a vasorelaxant in the porcine pulmonary arterial preparations (Zellers et al., 1994), the contractions to leukotriene C_4 were inhibited by indomethacin whereas the leukotriene D_4 contractions were unchanged (present report). This observation supports that a balance between different cyclooxygenase products, rather than one specific metabolite, regulates the contractions to cysteinyl-leukotrienes. Isolated human pulmonary arteries also release prostacyclin after cysteinyl-leukotriene stimulation (Bäck et al., 2000). However, in the human preparations a potentiation of the contractions to leukotriene C_4 is observed after either indomethacin treatment or endothelial rubbing. A similar difference between human and porcine pulmonary arteries has previously been reported for endothelium-dependent responses to acetylcholine, where vasorelaxant prostanoids are important in the human pulmonary arteries whilst in porcine pulmonary arteries vasoconstrictor prostanoids dominate (Lawrence et al., 1998).

A definitive explanation for the observation (present report) that indomethacin inhibited the leukotriene C_4 but not the leukotriene D_4 contractions is currently not available. Leukotriene C_4, but not leukotriene D_4, may lead to the release of an additional contractile cyclooxygenase pathway metabolite other than thromboxane A_2. The finding that leukotriene C_4 induced greater contractions than leukotriene D_4 in endothelial intact preparations, whereas the two cysteinyl-leukotrienes were equipotent in rubbed preparations (present report) support that there is a contractile factor predominately released by leukotriene C_4 and that this factor is endothelial in origin. This further implicates a mediator other than thromboxane A_2, since thromboxane B_2 was released also from rubbed preparations (present report). The antagonists of $CysLT_1$ and $CysLT_2$ receptors did not alter the increased production of 6-keto prostaglandin $F_{1\alpha}$ and thromboxane B_2 after cysteinyl-leukotriene stimulation, whereas the functional results indicated that the CysLT receptor antagonists inhibited the endothelial CysLT receptors associated with the putative release of contractile factors (present report). This indicates that different CysLT receptors may be involved in the modulatory part of the contractile responses to cysteinyl-leukotrienes in the porcine pulmonary artery.

The present report shows that the major part of the cysteinyl-leukotriene contraction is directly mediated via a CysLT receptor on the smooth muscle, resistant to the known antagonists, and that the roles of endothelial CysLT receptors and cyclooxygenase products are comparatively small and modulatory. Although the composition and action of those modulatory factors seem to differ between porcine and human pulmonary arteries (present report; Lawrence et al., 1998; Bäck et al., 2000), both the agonist and antagonist profiles of the CysLT receptors on the vascular smooth muscles of those two tissues are closely similar (Bäck et al., 2000). Therefore, the porcine pulmonary artery may represent a relevant model for the human.

In conclusion, the resistance of the cysteinyl-leukotriene contractions of the vascular smooth muscle to both $CysLT_1$ and $CysLT_2$ receptor antagonism suggests the presence of a single receptor subtype different from the previously described $CysLT_1$ and $CysLT_2$ receptors. This notion is further supported by the observations with leukotriene E_4, namely that this agonist failed to contract the porcine pulmonary arterial smooth muscle and did not modify the responses to either leukotriene C_4 or D_4. Presently, the pharmacological assessment of the CysLT receptors is compromised until more selective antagonists are made available. The pig pulmonary arterial preparation represents a pertinent model for the development of compounds with CysLT receptor antagonistic effects since the response represents a substantial functional muscle contraction (approximately 1.5 g), is reproducible and easily monitored. The $CysLT_1$ receptor was recently cloned (Lynch et al., 1999; Sarau et al., 1999) and in the search for the molecular structure of other CysLT receptors, the porcine pulmonary artery may be a useful tool since in rubbed preparations, the only functional CysLT receptor present is different from the earlier described $CysLT_1$ and $CysLT_2$ receptors.

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