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Pharmacological evidence for a novel cysteinyl-leukotriene receptor subtype in human pulmonary artery smooth muscle

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1 To characterize the cysteinyl-leukotriene receptors (CysLT receptors) in isolated human pulmonary arteries, ring preparations were contracted with leukotriene C_4 (LTC_4) and leukotriene D_4 (LTD_4) in either the absence or presence of the selective $CysLT_1$ receptor antagonists, ICI 198615, MK 571 or the dual $CysLT_1$/$CysLT_2$ receptor antagonist, BAY u9773.

2 Since the contractions induced by the cysteinyl-leukotrienes (cysLTs) in intact preparations failed to attain a plateau response over the concentration range studied, the endothelium was removed and the tissue treated continuously with indomethacin (Rubbed + INDO). In these latter preparations, the pEC_{50} for LTC_4 and LTD_4 were not significantly different (7.61 ± 0.07, $n=20$ and 7.96 ± 0.09, $n=22$, respectively). However, the LTC_4 and LTD_4 contractions were markedly potentiated when compared with data from intact tissues.

3 Leukotriene E_4 (LTE_4) did not contract human isolated pulmonary arterial preparations. In addition, treatment of preparations with LTE_4 (1 μM; 30 min) did not modify either the LTC_4 or LTD_4 contractions.

4 Treatment of preparations with the S-conjugated glutathione (S-hexyl-GSH; 100 μM, 30 min), an inhibitor of the metabolism of LTC_4 to LTD_4, did not modify LTC_4 contractions.

5 The pEC_{50} values for LTC_4 were significantly reduced by treatment of the preparations with either ICI 198615, MK 571 or BAY u9773 and the pK_B values were: 7.20, 7.02 and 6.26, respectively. In contrast, these antagonists did not modify the LTD_4 pEC_{50} values.

6 These findings suggest the presence of two CysLT receptors on human pulmonary arterial vascular smooth muscle. A $CysLT_1$ receptor with a low affinity for $CysLT_1$ antagonists and a novel CysLT receptor subtype, both responsible for vasoconstriction. Activation of this latter receptor by LTC_4 and LTD_4 induced a contractile response which was resistant to the selective $CysLT_1$ antagonists (ICI 198615 and MK 571) as well as the non-selective ($CysLT_1$/$CysLT_2$) antagonist, BAY u9773.

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Abbreviations: LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; E_{max}, maximal contraction; cysLTs, cysteinyl-leukotrienes; CysLT receptors, cysteinyl-leukotriene receptors; INDO, indomethacin

Introduction

Cysteinyl-leukotrienes (cysLTs), products of the 5-lipoxygenase enzymatic pathway, are metabolites of arachidonic acid and are potent constrictor agents in a number of vascular beds (Dahlén *et al*., 1982; Smedegard *et al*., 1982; Berkowitz *et al*., 1984; Piper *et al*., 1985). Unfortunately, the characterization and identification of the CysLT receptors responsible for the vasoconstriction in a number of vascular beds has received little attention.

Presently, there is evidence for two functional CysLT receptor subtypes in the human lung, a $CysLT_1$ (Buckner *et al*., 1986) and $CysLT_2$ (Labat *et al*., 1992). The former is antagonized by a number of classical selective antagonist while the latter is resistant to all of these compounds except for BAY u9773 which is not a selective antagonist (Gorenne *et al*., 1996). Recently, a cDNA encoding a $CysLT_1$ receptor was cloned (Lynch *et al*., 1999; Sarau *et al*., 1999) and subsequently the clonage of the $CysLT_2$ receptor was also reported (Takasaki *et al*., 2000; Heise *et al*., 2000; Nothacker *et al*., 2000). These molecular studies have confirmed the initial observations that two CysLT receptors exist (Drazen *et al*., 1980; Krell *et al*., 1981; Fleisch *et al*., 1982; Buckner *et al*., 1986; Labat *et al*., 1992). However, evidence from functional (Tudhope *et al*., 1994; Bäck *et al*., 2000a, b) and radioligand binding studies (Ravasi *et al*., 2000) as well as molecular investigations (Mellor *et al*., 2001) demonstrated that another CysLT receptor may also exist.

Since Bäck *et al*. (2000a) have suggested the presence of another CysLT receptor in human isolated pulmonary arterial preparations, the aim of this investigation was to further characterize the CysLT receptor in this tissue and to provide pharmacological evidence of those CysLT receptors

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responsible for vasoconstriction in the human pulmonary vascular bed.

Methods

Tissue preparation

The lung samples were obtained from patients (23 male and two female) who had undergone surgery for lung carcinoma. The mean age was 64±2 years. Intrapulmonary arteries and veins were removed, dissected free from surrounding tissue and cut into rings with a length of 3–5 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. Experiments were performed on preparations with an endothelium present (Intact) and on rings where the endothelium was mechanically removed by gently rubbing the luminal surface with a metal forceps. These latter preparations were continuously exposed to indomethacin (1.7 μM; Rubbed + INDO). Changes in force were recorded using isometric force displacement transducers (Narco F-60) connected to Linseis physiographs. The responses were monitored with an EMKA IOX data acquisition system. The preparations were placed in 10 ml organ baths under an initial load of 1.5 g and allowed to equilibrate for 90 min with washes every 10 min.

Contractions

Subsequent to the equilibration period, the intact arterial preparations were incubated 30 min with Tyrode's solution and cysLT concentration-effect curves were produced. The preparations (Rubbed + INDO), were exposed to either Tyrode's solution or Tyrode's solution containing a CysLT antagonist (ICI 198615 at 1 μM; MK 571 at 1 μM or BAY u9773 at 3 μM; 30 min) or S-hexyl-GSH (100 μM; 30 min). In addition, LTC_4 concentration-effect curves were produced in rings (Rubbed + INDO) treated with ICI 198615 (3 μM; five preparations from two lung samples). When the cysLT contraction induced by the highest concentration had attained a plateau, the preparations were challenged with norepinephrine (10 μM). The response to norepinephrine (10 μM) in intact preparations was: 2.45±0.40 *g* (n=20). In preparations (Rubbed + INDO), the contraction induced by norepinephrine (10 μM) was: 2.65±0.32 *g* (n=22).

In a limited number of experiments BAY u9773 (3 μM; n=3) was added to isolated intact human pulmonary arteries and veins at resting tone.

Data analysis

The contractions are expressed as increased tension in grams or as per cent of the norepinephrine response. The maximal contraction (E_{max} value) produced with the highest concentration of the agonist and the half-maximal effective concentration value (EC_{50} value) were interpolated from the individual concentration-effect curves. The pEC_{50} values were calculated as the negative log of the EC_{50} values. When the pEC_{50} values obtained in the presence and absence of the antagonist were significantly different, the equilibrium dissociation constant for the antagonist (K_B value) was calculated. The following equation was used: $K_B = [B]/(DR-1)$, where [B] is the concentration of the antagonist and DR (dose ratio) is the ratio of the EC_{50} of agonist in the presence and absence of antagonist. The pK_B values were calculated as the negative log of the K_B values. All data are expressed as means±s.e.means. Statistical evaluation was performed using a Student's *t*-test for paired or unpaired data, a *P*-value of less than 0.05 was considered significant.

Compounds

Norepinephrine, indomethacin (INDO), and S-hexyl glutathione (S-hexyl GSH) were obtained from Sigma (St. Louis, MO, U.S.A.). LTC_4, LTD_4, and BAY u9773 (6(*R*)-(4′-carboxyphenylthio) - 5(*S*)-hydroxy - 7(*E*),9(*E*),11(*Z*)14(*Z*)-eicosatetrenoic acid) were from Cayman Chemicals (Reading, U.K.). ICI 198615 {[1-[[2-methoxy-4-[[(phenyl-sulfonyl)amino]carbonyl]-phenyl]methyl]-1H-indazol-6-yl]-carbamic acid cyclopentyl ester} was from Zeneca (Wilmington, DE, U.S.A.) and MK 571 ((3-(2(7-chloro-2-quinolinyl)ethenyl)-phenyl)(3-(dimethylamino-3-oxopropyl)thio)methyl)thio propanoic acid) was from Bayer (U.K.).

Solutions of cysLTs and BAY u9773 were obtained by diluting stock solutions at concentrations of 1 mM (LTC_4 and LTE_4), 0.5 mM (LTD_4) and 10 mM (BAY u9773) into Tyrode's solution. INDO was dissolved in 1% ethanol in Tyrode's solution, ICI 198615 in dimethyl sulphoxide (DMSO; the final bath concentration of the solvents being less than 0.1%). Norepinephrine was dissolved in Tyrode's solution.

Results

Intact human isolated pulmonary arterial preparations contracted when challenged with cysLT. However, the concentration-effect curves did not attain a plateau response over the concentration range studied (Figure 1). In contrast, the results presented in Figure 1 show that the contractile response to LTC_4 and LTD_4 were markedly increased in human pulmonary arterial preparations where the endothelium had been removed and the tissues treated with indomethacin (Rubbed + INDO). Under these latter conditions LTE_4 did not contract human pulmonary arterial preparations. In addition, the LTC_4 concentration effect curves were not altered by S-hexyl GSH (inhibitor of LTC_4 metabolism; Figure 2).

The antagonist BAY u9773 (3 μM) was examined on basal tone in intact pulmonary arterial and venous preparations (Figure 3). An increase in basal tone was observed in human pulmonary veins whereas in arteries no contractions were observed. The data presented in Figure 4 show that the classical $CysLT_1$ antagonists, ICI 198615 (1 μM), MK 571 (1 μM) and the non-selective dual antagonist ($CysLT_1$/$CysLT_2$), BAY u9773 (3 μM) shifted the LTC_4 curves to the right and the pEC_{50} values were significantly different (Table 2). However, a higher concentration of ICI 198615 (3 μM; five preparations two different lung samples) did not cause a further displacement of the LTC_4 curves (data not shown). In contrast, these same antagonists failed to block the

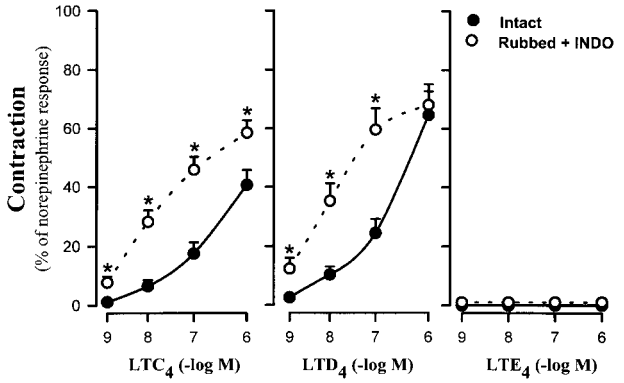

Figure 1 Cysteinyl-leukotriene concentration-effect curves produced in human isolated pulmonary arterial preparations. The results are derived from intact tissues (endothelium present, solid circle) and tissues devoid of an endothelium and treated with indomethacin (Rubbed + INDO, empty circle). Contractions were expressed as per cent of norepinephrine (10 μM) contraction and values are means ± s.e.means (see Table 1 for the number of lung samples for LTC_4 and LTD_4). LTE_4 data were derived from four different lung samples. *Indicates values significantly different from results in intact preparations when analysed for each concentration (Student's *t*-test; $P<0.05$).

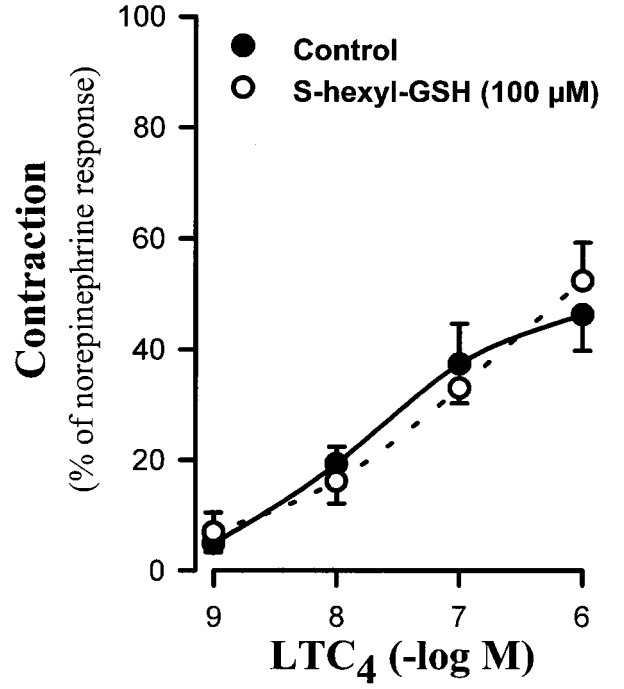

Figure 2 The effects of S hexyl glutathione (S-hexyl-GSH) on LTC_4 concentration effect curves in human isolated pulmonary vascular preparations devoid of an endothelium and treated with indomethacin (Rubbed + INDO). Control and results obtained in tissues treated with S-hexyl-GSH (100 μM, 30 min) are presented. Values are means ± s.e. means in paired preparations from three different lung samples. Contractions were expressed as per cent of norepinephrine (10 μM) contraction.

contractions induced by LTD_4 (Figure 5). The pK_B values for the three antagonists are presented in Table 2. However, MK 571 (1 μM) significantly reduced the LTD_4 contractions at the highest agonist concentrations (0.1 and 1 μM) but did not

Table 1 Effects of cysteinyl-leukotrienes in human isolated pulmonary arterial preparations

Preparation	n	*pEC_{50} value*	*E_{max} (%)*
LTC_4 contractions			
Intact	15	NC	41 ± 5
Rubbed + INDO	20	7.61 ± 0.07	59 ± 4
LTD_4 contractions			
Intact	5	NC	65 ± 8
Rubbed + INDO	22	7.96 ± 0.09	68 ± 7

All values are expressed as means ± s.e.means. The number of lung samples from different patients (*n*) are shown. Data from Intact preparations (endothelium present) and pulmonary arteries where the endothelium was mechanically removed and the tissues were exposed continuously to INDO (1.7 μM; Rubbed + INDO) are presented. The pEC_{50} values (−log M of EC_{50} value) and E_{max} (maximal contraction obtained with the highest concentration of each agonist expressed as per cent of norepinephrine (10 μM)). NC indicates not calculated since no plateau was observed.

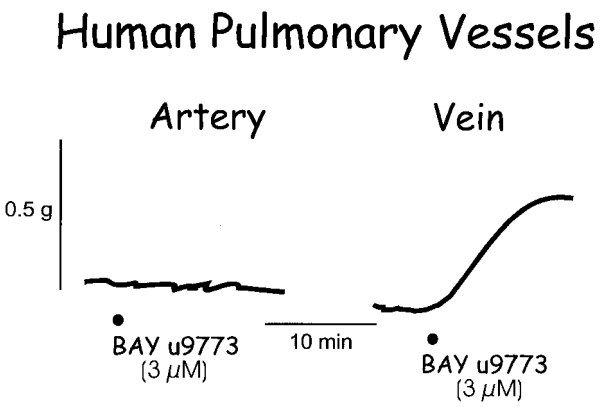

Figure 3 The effects of BAY u9773 on basal tone in intact human isolated pulmonary vascular preparations. Results of a representative experiment are shown ($n=3$ lung samples).

modify the pEC_{50} values (Table 2). In addition, exposure of human pulmonary arterial preparations to LTE_4 (1 μM; 30 min; $n=4$) did not significantly modify either the LTC_4 or LTD_4 response (Figure 6). The pEC_{50} values for LTC_4 (7.66 ± 0.33) and LTD_4 (7.72 ± 0.07) were not altered subsequent to this LTE_4 treatment in paired lung samples (LTC_4, 7.55 ± 0.18 and LTD_4, 7.44 ± 0.12).

Discussion

Bäck *et al.* (2000a) observed that the LTC_4 contractions in human pulmonary arteries were not blocked by either the $CysLT_1$ antagonist, MK 571, or the non-selective $CysLT_1$/$CysLT_2$ antagonist, BAY u9773. These preliminary data suggested that the CysLT receptor responsible for the contractions did not fit the classical CysLT receptor profile since the responses were resistant to the known CysLT antagonists. The data (present report) provide further information on this CysLT receptor. However, the results suggest that the cysLT contractions of human isolated pulmonary arteries are more complex than what was initially

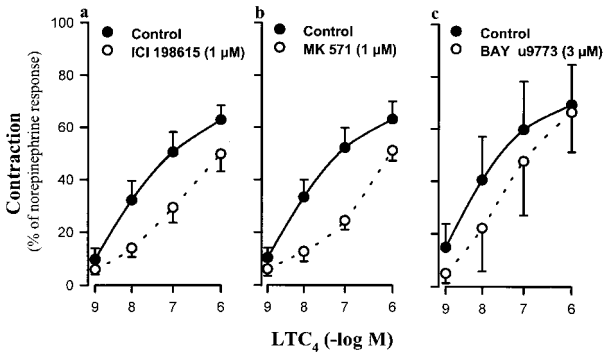

Figure 4 LTC_4 concentration-effect curves produced in human isolated pulmonary arterial preparations devoid of an endothelium and treated with indomethacin (Rubbed + INDO). Control and results obtained in tissues treated with (a) ICI 198615 (1 μM, 30 min), panel (b) MK 571 (1 μM, 30 min), and panel (c) BAY u9773 (3 μM, 30 min). Values are means ± s.e.means (see Table 2 for significance and number of preparations used). Contractions were expressed as per cent of norepinephrine (10 μM) contraction.

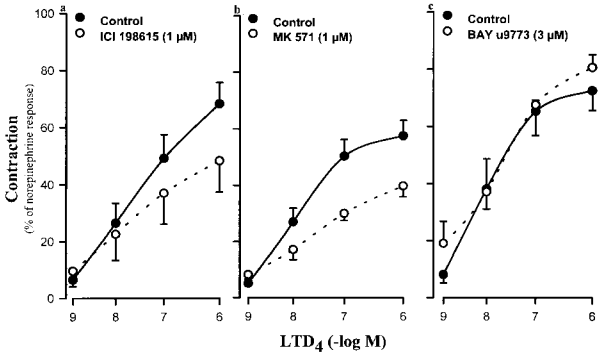

Figure 5 LTD_4 concentration-effect curves produced in human isolated pulmonary arterial preparations devoid of an endothelium and treated with indomethacin (Rubbed + INDO). Control and results obtained in tissues treated with (a) ICI 198615 (1 μM, 30 min), (b) MK 571 (1 μM, 30 min), and (c) BAY u9773 (3 μM, 30 min). Values are means ± s.e.means (see Table 2 for significance and number of preparations used). Contractions were expressed as per cent of norepinephrine (10 μM) contraction.

Table 2 Effects of antagonists on cysteinyl-leukotriene contractions in human isolated pulmonary arterial preparations

Treatment	n	*pEC_{50} value* (−log M)	*E_{max}* (%)	*pK_B value*
LTC_4 contractions				
Tyrode	7	7.91 ± 0.19	63 ± 6	
ICI 198615 (1 μM)	7	7.20 ± 0.42*	50 ± 6	7.20 ± 0.38
Tyrode	8	7.98 ± 0.16	63 ± 7	
MK 571 (1 μM)	8	6.91 ± 0.33*	51 ± 4	7.02 ± 0.36
Tyrode	6	8.16 ± 0.39	69 ± 8	
BAY u9773 (3 μM)	6	7.51 ± 0.47*	63 ± 13	6.26 ± 0.26
LTD_4 contractions				
Tyrode	5	7.85 ± 0.16	57 ± 7	
ICI 198615 (1 μM)	5	7.74 ± 0.38	49 ± 11	NS
Tyrode	7	7.89 ± 0.11	58 ± 5	
MK 571 (1 μM)	7	7.92 ± 0.24	40 ± 4*	NS
Tyrode	4	8.03 ± 0.16	73 ± 7	
BAY u9773 (3 μM)	4	7.93 ± 0.36	81 ± 5	NS

Data are from pulmonary arteries devoid of an endothelium and treated with indomethacin (Rubbed + INDO). All values are expressed as means ± s.e.means. The number of lung samples from different patients (n) are presented and * indicates values significantly different ($P<0.05$) compared with data from Tyrode paired preparations. The pK_B values were calculated when there was a significant shift in the pEC_{50} value following antagonist treatment (Student's t-test: $P<0.05$). NS indicates no significant effect of antagonist.

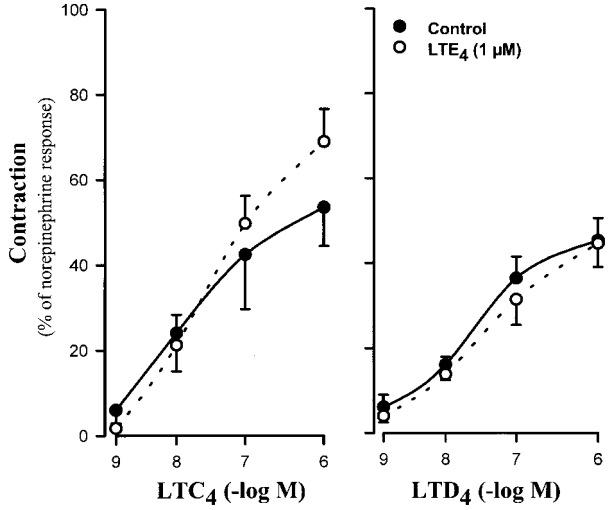

Figure 6 LTC_4 and LTD_4 concentration-effect curves produced in human isolated pulmonary arterial preparations devoid of an endothelium and treated with indomethacin (Rubbed + INDO). Control and results obtained in tissues treated with LTE_4 (1 μM, 30 min). Values are means ± s.e.means in paired preparation from four lung samples. Contractions were expressed as per cent of norepinephrine (10 μM) contraction.

proposed. The evidence demonstrates that there are factors released from both the endothelium as well as the vascular smooth muscle which modify the cysLTs contractions. In the present investigation the production of cysLT concentration-effect curves was established by treatment of the human pulmonary arterial preparations devoid of an endothelium with indomethacin. Under these experimental conditions, pEC_{50} values were calculated and established that LTC_4 and LTD_4 were equipotent contractile agonists in human isolated pulmonary arterial preparations whereas LTE_4 did not contract these tissues.

Previous reports (Hanna *et al*., 1981; Schellenberg & Foster, 1984; Bourdillat *et al*., 1987) have demonstrated that human isolated pulmonary arteries exhibited only a small response when stimulated by cysLTs. These observations were recently confirmed by Bäck *et al*. (2000a) using LTC_4. The present report indirectly suggests that the reason why cysLT concentration-effect curves were not produced in these previous reports was related to the release of endogenous relaxant factors which modulate the vascular responses (Figure 1). The results (present report) demonstrate that both LTC_4 and LTD_4 contractions were enhanced when the endothelium was removed and the preparations were treated with indomethacin. These observations suggest that the failure to produce a concentration-effect curve in intact

human pulmonary arterial preparations (endothelium present) over the concentration ranged used was due to the release of endothelium derived relaxant factors. However, the relaxant factors released from the human isolated pulmonary artery may, in part, also originate from the vascular smooth muscle since either indomethacin treatment or removal of endothelium previously has been shown to only partially modify the contractions (Bäck *et al*., 2000a), whereas the combination of indomethacin treatment and removal of the endothelium unmasked the full cysLTs concentration-effect curves in these tissues (present report). While the nature and origin of the relaxant factor remains to be elucidated the data indirectly suggest that a cyclo-oxygenase metabolite may be involved. Previous reports have demonstrated that prostacyclin (PGI_2) may be released from the endothelium in arterial preparations (Moncada *et al*., 1977) but there is little information concerning release of this metabolite from vascular smooth muscle. However, in a recent report Soler *et al*. (2000) have shown that human vascular smooth muscle cells in culture express prostaglandin E synthase and demonstrated that PGE_2 and prostacyclin were produced, during basal, and enhanced after stimulation with a variety of agonists. Although these results were derived from the human popliteral artery, other investigators (Jourdan *et al*., 1997) had already shown that human pulmonary artery vascular smooth muscle cells in culture produced these metabolites and these observations have been confirmed by Shaul *et al*. (1999). Together these results suggest that the vascular smooth muscle may be responsible for the release of cyclooxygenase metabolites which may modify the vascular contractions (Mugridge *et al*., 1984; Qian *et al*., 1994; Walch *et al*., 1999, 2001).

LTC_4 is known to be metabolized to LTD_4 as has been reported in other preparations (Krell *et al*., 1981; Jones *et al*., 1984; Bäck *et al*., 2001). Since LTC_4 and LTD_4 curves were similar in human isolated pulmonary arteries (Bäck *et al*., 2000a) one explanation may be that the LTC_4 was metabolized to LTD_4. However, S-hexyl-GSH did not modify the LTC_4 concentration-effect curves in human pulmonary arterial preparations (Figure 4) suggesting that the contractile effects were directly dependent on LTC_4. These data show that in human airways (Buckner *et al*., 1986) and human vascular smooth muscle preparations (Labat *et al*., 1992; present report) the cysLT contractions were not modified by the cysLT metabolic enzyme inhibitors suggesting that major differences in enzymatic activities may exist between species (Bäck *et al*., 2001). In addition, under the present experimental conditions (rubbed preparations treated with indomethacin), LTC_4 and LTD_4 were equipotent. These results suggest a major difference in the rank order potency of these contractile agonists between human airways ($LTC_4 \geqslant LTD_4 \geqslant LTE_4$; Buckner *et al*., 1986; Labat *et al*., 1992) and human pulmonary veins ($LTC_4 = LTD_4 > LTE_4$; Labat *et al*., 1992) when compared with the contractions obtained in human pulmonary arteries ($LTC_4 = LTD_4$). Previous attempts to establish the cysLT potency in human isolated pulmonary arterial preparations have failed (Bourdillat *et al*., 1987) or provide, at best, only estimates (Schellenberg & Foster, 1984; Bäck *et al*., 2000a).

The observation that LTD_4 contractions (present report) were not modified by either MK 571, ICI 198615 or BAY u9773 extend the observation of Bäck *et al*. (2000a) to include the equipotent ligand, LTD_4. Thus LTD_4 induced contractions are also resistant to the three cysLT antagonists, results which are similar to what had been previously reported for LTC_4. Several reports (Labat *et al*., 1992; Ortiz *et al*., 1995) have demonstrated that LTC_4 and LTD_4 contractions produced in human isolated pulmonary veins were resistant to ICI 198615 and MK 571 but were antagonized by BAY u9773. These observations lead to the suggestion that a $CysLT_2$ receptor was present in the human pulmonary veins and the existence of a $CysLT_2$ receptor has recently been confirmed by molecular techniques (Nothacker *et al*., 2000; Heise *et al*., 2000; Takasaki *et al*., 2000). However, in human pulmonary arterial preparations, the LTD_4 contractions were resistant not only to ICI 198615 and MK 571 but also to BAY u9773 suggesting that the receptor present in the pulmonary arterial vascular smooth muscle may be different from that in the pulmonary veins. Together these data support the notion that the ligands may be acting at the same CysLT receptor and that this receptor is not identical to that responsible for contraction of human airways (blocked by $CysLT_1$ antagonists) nor the receptor associated with the contraction of human pulmonary veins ($CysLT_2$). Therefore, the observation (present report) that LTD_4 contractions were resistant to all three antagonists suggests the presence of another CysLT receptor subtype in the human lung.

Bäck *et al*. (2000a) previously demonstrated that LTC_4-induced contractions were not modified by the antagonists, however, the results presented in Table 2 demonstrate that the three antagonists significantly modified the LTC_4 pEC_{50} values. This apparent discrepancy between the initial publication (Bäck *et al*., 2000a) and the present work warrants an explanation. The experimental conditions were different. The optimal conditions to obtain cysLT concentration-effect curves is in tissues where the endothelium had been removed and the tissues treated with indomethacin (present report). Under these conditions a pharmacological assessment of the antagonists can be performed on the agonist concentration-effect curve. Thus inhibition of a cyclooxygenase metabolite at the level of the vascular smooth muscle which masks the cysLT contractions is a prerequisite for uncovering the $CysLT_1$ receptor component in human isolated pulmonary arterial muscle preparations. Under the previous experimental conditions (Bäck *et al*., 2000a), namely, intact tissues, endothelium denuded tissues or intact tissues treated with indomethacin only the antagonist resistive contractile component is observed. Interestingly, the results obtained with the $CysLT_1$ antagonists in human pulmonary arteries (Table 2) were at least one order of magnitude different from that observed in human airways (ICI 198615, $pK_B = 8.3$; MK 571, $pK_B = 8.8$; Gorenne *et al*., 1996) suggesting that the $CysLT_1$ receptor in the human pulmonary artery may have a lower affinity than those present in the airways. Of considerable interest is the observation that LTC_4 is blocked by the classical antagonists but not LTD_4 suggesting again that the $CysLT_1$ receptor activated by LTC_4 does not fit the classical profile.

Recently, BAY u9773 has been reported to be a partial agonist in cells containing the $CysLT_2$ cloned receptor (HPN321; Nothacker *et al*., 2000). In addition, in human pulmonary veins BAY u9773 has partial agonist contractile activities at the $CysLT_2$ receptor (Labat *et al*., 1992).

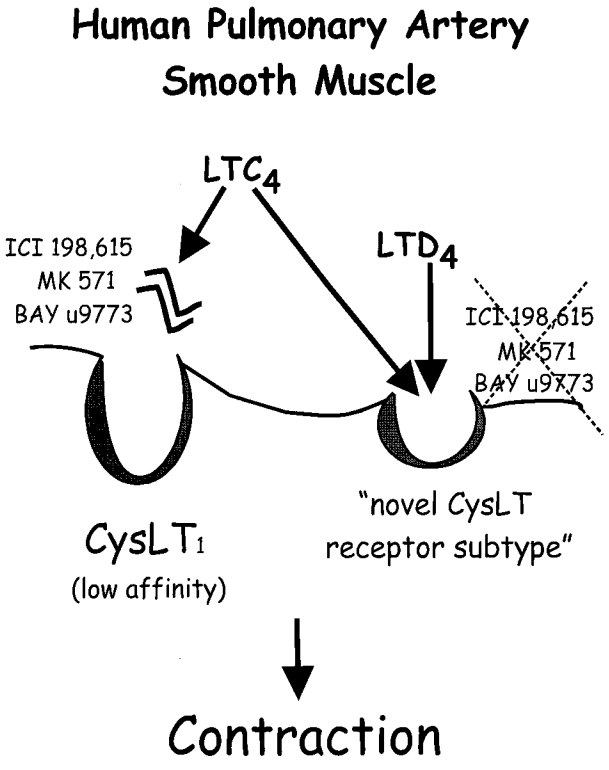

Figure 7 A schematic presentation of the CysLT receptors present in human pulmonary arterial vascular smooth muscle. LTC_4 activates two receptors in arterial preparations. One receptor has a low affinity for the selective $CysLT_1$ antagonists (ICI 198615 and MK 571) and is antagonized by the dual antagonist (BAY u9773). Another 'novel CysLT receptor subtype' is activated only by LTC_4 and LTD_4 and the contractions are not blocked by any of these antagonists.

However, the observations concerning the effects of BAY u9773 on basal tone in intact pulmonary arterial preparations showed that this compound did not constrict these vascular preparations. The differential effects in arteries and veins observed with the analogue of LTE_4 (BAY u9773) were also observed with LTE_4. Thus in human pulmonary arteries no contractile activity was observed with either of these compounds, providing evidence for the lack of $CysLT_2$ receptors in arterial preparations. Furthermore, LTE_4 did not block the cysLT contractions in human isolated arterial preparations. In contrast, BAY u9773 significantly reduced the LTC_4 pEC_{50} values without altering the LTD_4 contractions. These results are markedly different from the LTE_4 and BAY u9773 antagonism observed in human airways and in human pulmonary veins (Labat *et al*.,1992). These observations provide further support for the existence of another CysLT receptor subtype in the human lung since there was antagonist divergence at the CysLT receptor in the human isolated pulmonary arteries.

Tudhope *et al*. (1994) provided initial evidence for the presence of another functional CysLT receptor and this observation has received support from a number of recent functional studies (Bäck *et al*., 2000a, b) and provide further evidence for a CysLT receptor which does not fit the classical CysLT receptor profile (Coleman *et al*., 1995). Of considerable interest is the report in human mast cells (Mellor *et al*., 2001) which demonstrated a partial antagonist effect of BAY u9773 on LTC_4 but not on LTD_4-mediated calcium flux. In addition, data from radioligand binding studies have also suggested the existence of another CysLT receptor (Ravasi *et al*., 2000) since BAY u9773 blocked the binding of LTC_4 but not that of LTD_4. These reports suggest a discriminative effect of LTC_4 and LTD_4 on a $CysLT_1$ receptor, evidence which is also provided by the present report.

In summary, the evidence presented in this report suggest the existence of several CysLT receptor subtypes in human pulmonary arteries which are responsible for vasoconstriction. While Bäck *et al*. (2000b) provided the initial observation for a different CysLT receptor subtype in the human pulmonary artery, the present report extend these data and provide pertinent evidence that this novel CysLT receptor subtype is activated by LTC_4 and LTD_4 and is resistant to both the selective and non selective CysLT receptor antagonists. This profile is different from the $CysLT_1$ and $CysLT_2$ receptors previously described in human airways (Buckner *et al*., 1986) and pulmonary veins (Labat *et al*., 1992). In addition, the results also suggest the presence of a $CysLT_1$ low affinity receptor present in the vascular muscle layer which is activated by LTC_4. This preliminary and indirect evidence is schematically presented in Figure 7. An exploration of these observations either using ligand binding, cloning and sequencing of the receptor mRNA or transfection of a functional protein will clearly establish the existence of other receptor subtypes.

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