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# Receptor preferences of cysteinyl-leukotrienes in the guinea pig lung parenchyma

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**Abstract**

Two cysteinyl-leukotriene receptors, $CysLT_1$ and $CysLT_2$ receptors, have been cloned, but the contractions to cysteinyl-leukotrienes in the guinea pig lung parenchyma have been reported to be resistant to $CysLT_2$ receptor antagonism and to be only partially inhibited by $CysLT_1$ receptor antagonism. The receptor preferences of the individual cysteinyl-leukotrienes (leukotriene $C_4$, $D_4$ and $E_4$) in the guinea pig lung parenchyma were studied in organ baths. $CysLT_1$ receptor antagonists competitively inhibited the contraction to leukotriene $E_4$, but exhibited only weak antagonism of contractions to leukotriene $C_4$ and $D_4$. In the presence of the cyclooxygenese inhibitor indomethacin and the nitric oxide synthase inhibitor $N^{\omega}$-nitro-L-arginine (L-NOARG), the $CysLT_1$ receptor antagonists did not further inhibit the leukotriene $D_4$-induced contraction. These results suggest that leukotriene $E_4$ solely activates a $CysLT_1$ receptor, and that the $CysLT_1$ receptor antagonist-resistant contraction to leukotriene $D_4$ and $C_4$ is mediated via another CysLT receptor. © 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* Cysteinyl-leukotriene receptor; Lung parenchyma, guinea-pig; Cyclooxygenase; Nitric oxide (NO)

## 1. Introduction

Two cysteinyl-leukotriene receptors, $CysLT_1$ and $CysLT_2$ receptors, have been cloned recently (Heise et al., 2000; Lynch et al., 1999) and selective $CysLT_1$ receptor antagonists have been established as a new treatment of asthma (Drazen et al., 1999). It has however also been reported that human and porcine pulmonary arteries have a $CysLT_1$ and $CysLT_2$ receptor antagonist-insensitive part of the contraction to leukotriene $C_4$ (Bäck et al., 2000a,b), suggesting the presence of further CysLT receptor subtypes.

The guinea pig lung parenchyma, which is one of the most sensitive preparations with respect to contractile effects of cysteinyl-leukotrienes, has also been proposed to contain a CysLT receptor different from the $CysLT_1$ and $CysLT_2$ receptor (Tudhope et al., 1994; Wikström Jonsson et al., 1998). The $CysLT_1$/$CysLT_2$ receptor antagonist BAYu9773 (6(*R*)-(4′ -carboxyphenylthio)-5(*S*)-hydroxy-7(*E*),9(*E*),11(*Z*)14(*Z*)-eicosatetrenoic acid) either not at all or only slightly inhibits the contraction to leukotriene $C_4$ and $D_4$ in the guinea pig lung parenchyma (Tudhope et al., 1994; Wikström Jonsson et al., 1998). In addition, $CysLT_1$ receptor antagonists have been reported to exert less antagonism of contractions to leukotriene $C_4$ and $D_4$ in this preparation compared with other preparations, such as the guinea pig trachea and ileum (Norman et al., 1987; Tudhope et al., 1994; Wikström Jonsson et al., 1998). However, an initial report using the $CysLT_1$ receptor antagonist ICI-198,615 (1-((2-methoxy-4-(((phenylsulfonyl)amino)carbonyl)-phenyl)methyl)-1*H*-indazol-6-yl)carbamic acid cyclopentyl ester) in the guinea pig lung parenchyma reported a $pA_2$ value of 9.5 against leukotriene $D_4$-induced contractions (Snyder et al., 1987).

What has been missing in the studies of the purported new CysLT receptor in the guinea pig lung parenchyma is conclusive pharmacological characterisation. In fact, both $CysLT_1$ and $CysLT_2$ receptors were firstly characterised using pharmacological techniques, having different preferences to cysteinyl-leukotrienes and their antagonists (Dahlén, 2000). These results have contributed to recognise the two cloned receptors as $CysLT_1$ and $CysLT_2$ receptors (Heise et al., 2000; Lynch et al., 1999).

The aim of this study was to pharmacologically characterise the purported new CysLT receptor in the guinea pig lung parenchyma by performing an overall assessment of how different $CysLT_1$ receptor antagonists affect the contractile responses to cysteinyl-leukotrienes. In addition, since it has

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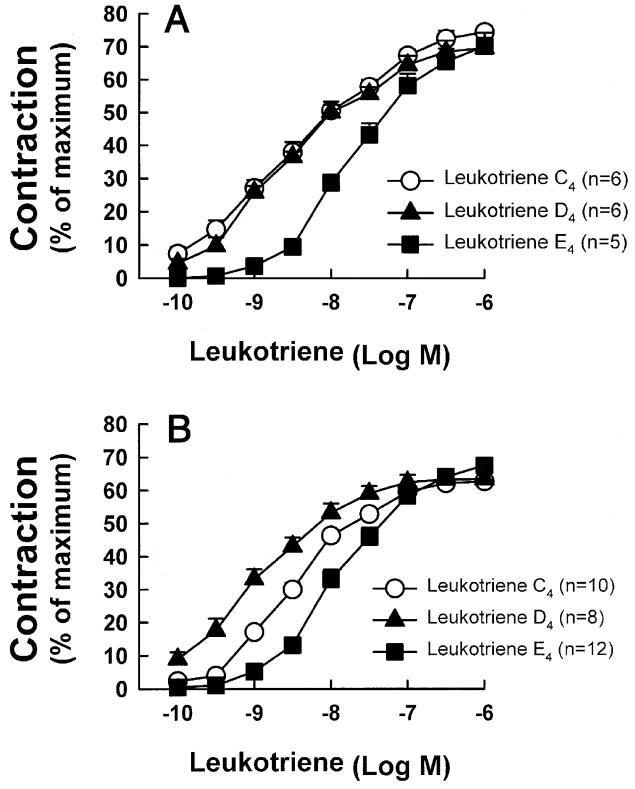


Fig. 1. Concentration–response curves for the contractile effects of leukotriene $C_4$, $D_4$ and $E_4$ in the guinea pig lung parenchyma in the absence (A) and presence (B) of L-serine borate (45 mM) and L-cysteine (5 mM). L-serine borate and L-cysteine were applied 30 min before the addition of leukotrienes. Each point represents the mean ± S.E.M, with the number of experiments indicated in parentheses.

been reported that the effects of cysteinyl-leukotrienes in the guinea pig lung parenchyma are partially mediated by cyclooxygenase products (Norman et al., 1987; Weichman et al., 1982), the effect of cyclooxygenase inhibition was also assessed.

## 2. Materials and methods

### 2.1. 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 experiments were approved by the local committee for animal experiments (N317/98).

### 2.2. Tissue bath experiments

Lung parenchymal preparations were placed in 5-ml organ baths containing Tyrode's solution (composition in 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 4 mN. Mechanical responses were recorded isometrically via Grass FT-03 force-displacement transducers connected to an EMKA data acquisition system (EMKA, Paris, France).

The bath solution was initially changed at 10-min intervals during a 90-min equilibration period. Tissue reactivity was assessed by cumulative challenge with histamine (0.3–30 μM). Leukotriene $C_4$, $D_4$ and $E_4$ were added as cumulative concentrations in each experiment. Drugs were administered 30 min prior to application of leukotrienes.

When the cysteinyl-leukotriene contraction reached a plateau at the end of the cumulative dosing, a maximal contraction was determined by simultaneous addition of histamine (1 mM), acetylcholine (1 mM) and KCl (40 mM). The contractions to histamine, acetylcholine and KCl were 3.5 ± 0.4 mN ($n=6$), 4.0 ± 0.7 mN ($n=6$) and 3.5 ± 0.5 mN ($n=5$) after challenge with leukotriene $C_4$, $D_4$ and $E_4$, respectively ($P>0.05$). In addition, the different treatments did not significantly alter the contractions to histamine, acetylcholine and KCl (data not shown).

### 2.3. Drugs

Acetylcholine, histamine, indomethacin, L-serine, boric acid, L-cysteine and $N^{\omega}$-nitro-L-arginine (L-NOARG) were obtained from Sigma (St. Louis, MO, USA). Leukotriene $C_4$, $D_4$ and $E_4$ were from Cascade Biochem (Reading, UK) or Cayman Chemicals (Ann Arbor, MI, USA). Zafirlukast was kindly provided from Astra Zeneca (Alderley, UK), MK-571 (3-(2-(7-chloro-2-quinolinyl)ethenyl)phenyl)((3-(dimethylamino-3-oxopropyl)thio)methyl)thio propanoic acid) was from Merck Frosst (Montreal, Canada), pobilukast was from Smith Kline Beecham (Swedeland, PA, USA), pranlukast was from Ono Pharmaceutical (Osaka, Japan).

L-serine borate was prepared from equimolar concentrations of L-serine and boric acid dissolved in distilled water and buffered at pH 7.4 with NaOH. L-cysteine, acetylcholine and histamine were dissolved in Tyrode's solution. Zafirlukast, pranlukast and pobilukast were dissolved in dimethylsulfoxide. Indomethacin was dissolved in 10% ethanol and 10% 1 M Tris (pH 8.0) in distilled water. MK-571 and KCl

Table 1
The p$D_2$ for the contraction to leukotriene $C_4$, $D_4$ and $E_4$ in the guinea pig lung parenchyma in the absence or presence of L-serine borate (45 mM) and L-cysteine (5 mM)

| | Control | $n$ | L-SeBo + L-cys | $n$ |
|---|---|---|---|---|
| Leukotriene $C_4$ | 8.21 ± 0.11 | 6 | 8.10 ± 0.07[a] | 10 |
| Leukotriene $D_4$ | 8.28 ± 0.08 | 6 | 8.70 ± 0.12[b] | 8 |
| Leukotriene $E_4$ | 7.41 ± 0.03[a,c] | 5 | 7.55 ± 0.09[a,c] | 12 |

Each data represents the mean ± S.E.M. of $n$ experiments. L-serine borate (L-SeBo, 45 mM) and L-cysteine (L-cys, 5 mM) were applied 30 min before the addition of leukotrienes.

[a] $P<0.05$ vs. leukotriene $D_4$.

[b] $P<0.05$ vs. control.

[c] $P<0.05$ vs. leukotriene $C_4$ (Tukey test).

were dissolved in distilled water. The final concentrations of ethanol or dimethylsulfoxide in the bath were always below 0.1%. Stock solutions of leukotriene $C_4$, $D_4$ and $E_4$ were kept in 50% ethanol in distilled water and concentrations were checked by UV-spectrometry.

### 2.4. Data analysis

All results are expressed as means ± S.E.M. Contractile responses are expressed as percent of the final contraction to histamine, acetylcholine and KCl. The maximal contraction

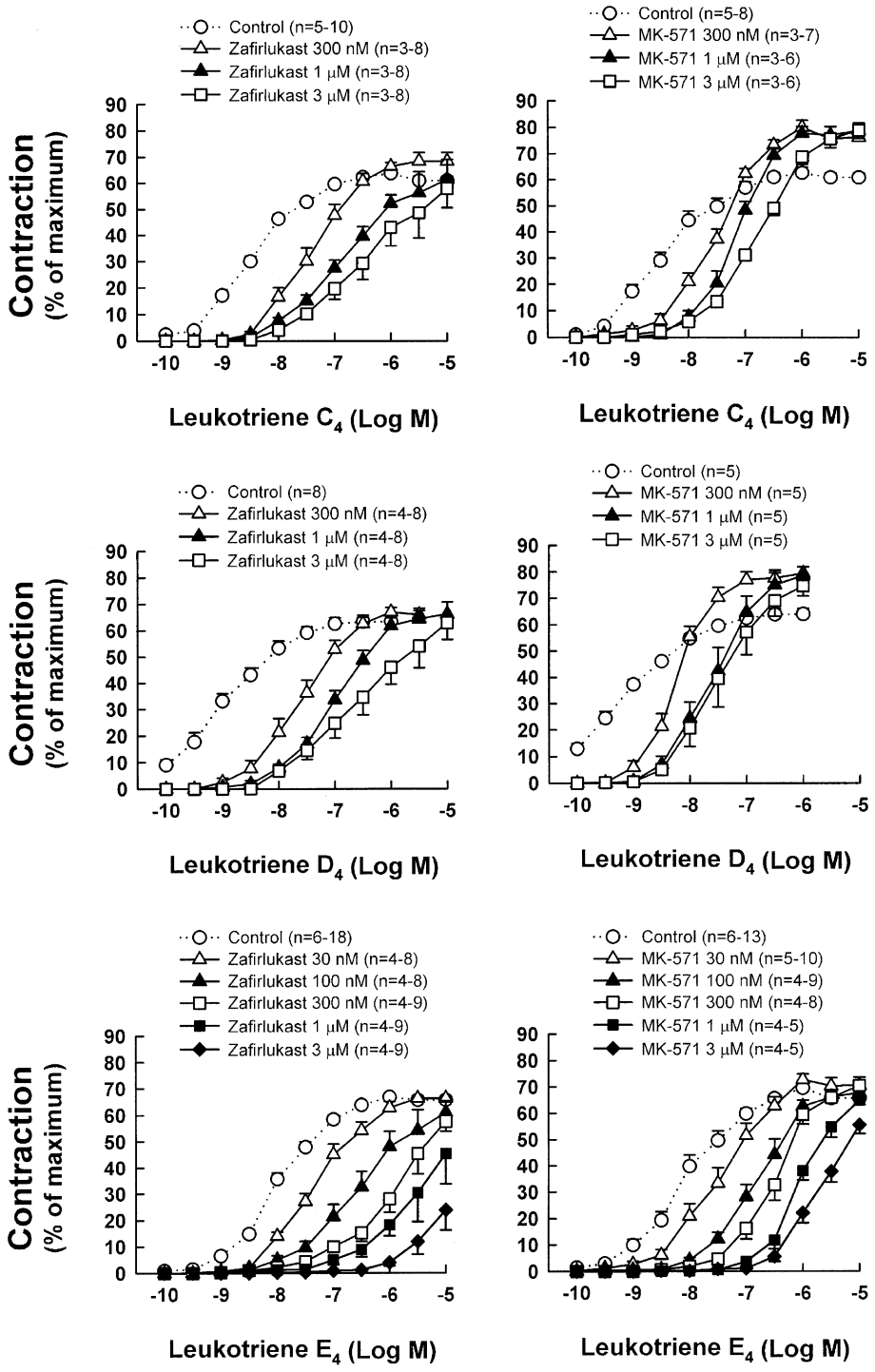


Fig. 2. Effects of the $CysLT_1$ receptor antagonists, zafirlukast and MK-571 on the contractions induced by cysteinyl-leukotrienes in the guinea pig lung parenchyma in the presence of L-serine borate (45 mM) and L-cysteine (5 mM). $CysLT_1$ receptor antagonists, L-serine borate and L-cysteine, were applied 30 min before the addition of leukotrienes. Each point represents the mean ± S.E.M, with the number of the experiments indicated in parentheses. Smaller number in parentheses indicates the number of experiments at high concentrations (3, 10 μM) of leukotrienes.

($E_{max}$) is expressed as the mean of the contractions induced by the highest concentration of the agonist in each individual concentration–response curve. The $EC_{50}$ was calculated by linear regression from each concentration–response curve. The p$D_2$ was calculated as the negative log of the $EC_{50}$. In experiments with $CysLT_1$ receptor antagonists, a dose ratio of $EC_{50}$ in the presence and absence of antagonists was calculated for each experiment relative to the paired control. The p$K_B$ was calculated for each antagonist concentration as the negative log of following equation: (antagonist concentration)/(dose ratio − 1). The p$A_2$ was determined as the mean of all p$K_B$ according to MacKay (1978). In addition, in order to confirm linearity, Schild plot analysis was performed on the means of the dose ratios (Arunlakshana and Schild, 1959). Statistically significant differences were determined by Student's *t*-test or one-way analysis of variances (ANOVA) followed by either Dunnett's or Tukey test, as appropriate. A $P$ value of less than 0.05 was considered significant.

## 3. Results

### 3.1. Effects of inhibitors of cysteinyl-leukotriene metabolism

In the absence of metabolic inhibitors for cysteinyl-leukotrines, leukotriene $C_4$, $D_4$ and $E_4$ contracted the guinea pig lung parenchyma (Fig. 1 and Table 1). The p$D_2$ for leukotriene $E_4$ were significantly lower than those for leukotriene $C_4$ and $D_4$ (Table 1) whereas there was no significant difference between the $E_{max}$ for leukotriene $C_4$ (74.1 ± 1.82%, $n=6$), $D_4$ (70.1 ± 2.91%, $n=6$) and $E_4$ (70.3 ± 4.10%, $n=5$). In the presence of the metabolic inhibitors, L-serine borate (45 mM) and L-cysteine (5 mM)(Bäck et al., 2001), the p$D_2$ for leukotriene $D_4$ were significantly higher than those for either leukotriene $C_4$ or $E_4$ and those for leukotriene $C_4$ were significantly higher than those for leukotriene $E_4$ (Fig. 1B and Table 1). The metabolic inhibitions significantly increased the p$D_2$ for leukotriene $D_4$ (Fig. 1 and Table 1).

Table 2
Effects of $CysLT_1$ receptor antagonists on the $E_{max}$ (%) induced by cysteinyl-leukotrienes in the guinea pig lung parenchyma in the presence of L-serine borate (45 mM) and L-cysteine (5 mM)

| Compound | nM | Leukotriene $C_4$ | *n* | Leukotriene $D_4$ | *n* | Leukotriene $E_4$ | *n* |
|---|---|---|---|---|---|---|---|
| Vehicle | | 62.8 ± 1.1 | 10 | 63.5 ± 2.4 | 8 | 66.9 ± 1.4 | 18 |
| Zafirlukast | 30 | | | | | 63.5 ± 2.4 | 8 |
| | 100 | | | | | 57.8 ± 3.9 | 8 |
| | 300 | 66.7 ± 1.4 | 8 | 67.3 ± 1.8 | 8 | | |
| | 1000 | 58.5 ± 3.2 | 8 | 65.3 ± 2.5 | 8 | | |
| Vehicle | | 62.8 ± 2.1 | 8 | 64.2 ± 2.1 | 5 | 69.7 ± 1.3 | 13 |
| MK-571 | 30 | | | | | 73.2 ± 2.2 | 10 |
| | 100 | | | | | 65.9 ± 2.4 | 9 |
| | 300 | 80.7 ± 2.5[a] | 7 | 79.6 ± 2.4[a] | 5 | 67.4 ± 2.2 | 8 |
| | 1000 | 80.3 ± 1.9[a] | 6 | 78.7 ± 3.4[a] | 5 | | |
| Vehicle | | | | 60.3 ± 4.6 | 4 | | |
| Pranlukast | 1000 | | | 79.1 ± 4.7[a] | 3 | | |
| Pobilukast | 1000 | | | 80.7 ± 1.6[a] | 3 | | |

$CysLT_1$ receptor antagonists, L-serine borate and L-cysteine were applied 30 min before the addition of leukotrienes. Each data represents the mean ± S.E.M. of *n* experiments.
[a] $P<0.05$ vs. Vehicle (Dunnett's test).

Table 3
Effects of $CysLT_1$ receptor antagonists on the p$D_2$ for the contraction to cysteinyl-leukotrienes in the guinea pig lung parenchyma in the presence of L-serine borate (45 mM) and L-cysteine (5 mM)

| Compound | nM | Leukotriene $C_4$ | *n* | Leukotriene $D_4$ | *n* | Leukotriene $E_4$ | *n* |
|---|---|---|---|---|---|---|---|
| Vehicle | | 8.44 ± 0.06 | 10 | 8.92 ± 0.07 | 8 | 7.95 ± 0.06 | 18 |
| Zafirlukast | 30 | | | | | 7.22 ± 0.13[a] | 8 |
| | 100 | | | | | 6.40 ± 0.18[a] | 8 |
| | 300 | 7.42 ± 0.13[a] | 8 | 7.60 ± 0.16[a] | 8 | | |
| | 1000 | 6.74 ± 0.12[a] | 8 | 6.96 ± 0.11[a] | 8 | | |
| Vehicle | | 8.41 ± 0.11 | 8 | 9.18 ± 0.11 | 5 | 8.01 ± 0.12 | 13 |
| MK-571 | 30 | | | | | 7.45 ± 0.16[a] | 10 |
| | 100 | | | | | 6.75 ± 0.12[a] | 9 |
| | 300 | 7.46 ± 0.10[a] | 7 | 8.18 ± 0.05[a] | 5 | 6.46 ± 0.12[a] | 8 |
| | 1000 | 7.12 ± 0.06[a] | 6 | 7.54 ± 0.14[a] | 5 | | |
| Vehicle | | | | 8.59 ± 0.19 | 4 | | |
| Pranlukast | 1000 | | | 7.36 ± 0.13[a] | 3 | | |
| Pobilukast | 1000 | | | 7.60 ± 0.20[a] | 3 | | |

$CysLT_1$ receptor antagonists, L-serine borate and L-cysteine were applied 30 min before the addition of leukotrienes. Each data represents the mean ± S.E.M. of *n* experiments.
[a] $P<0.05$ vs. Vehicle (Dunnett's test).

### 3.2. Effects of $CysLT_1$ receptor antagonists on cysteinyl-leukotriene-induced contractions

In the presence of metabolic inhibitors for cysteinyl-leukotrienes, pretreatment with one of the two $CysLT_1$ receptor antagonists, zafirlukast or MK-571, inhibited the concentration–response curves for leukotriene $E_4$ in a competitive manner, with p$A_2$ of 8.34 ± 0.07 (Fig. 3C) and 7.95 ± 0.09 (Fig. 3D), respectively. MK-571 significantly enhanced the $E_{max}$ for both leukotriene $C_4$ and $D_4$, whereas zafirlukast did not (Fig. 2 and Table 2). Zafirlukast significantly decreased the p$D_2$ for either leukotriene $C_4$ or $D_4$ without affecting the $E_{max}$, whereas MK-571 significantly decreased the p$D_2$ and increased the $E_{max}$ (Fig. 2, Tables 2 and 3). The slope of the Schild plot for zafirlukast against leukotriene $D_4$ did not approach unity (1.34, Fig. 3B). The p$A_2$ of zafirlukast for leukotriene $C_4$ (7.55 ± 0.11, Fig 3A) was significantly lower than those for leukotriene $E_4$ (8.34 ± 0.07, Fig 3C). Two other $CysLT_1$ receptor antagonists, pranlukast and pobilukast, also significantly decreased the p$D_2$ and increased the $E_{max}$ for leukotriene $D_4$ (Fig. 4 and Tables 2 and 3).

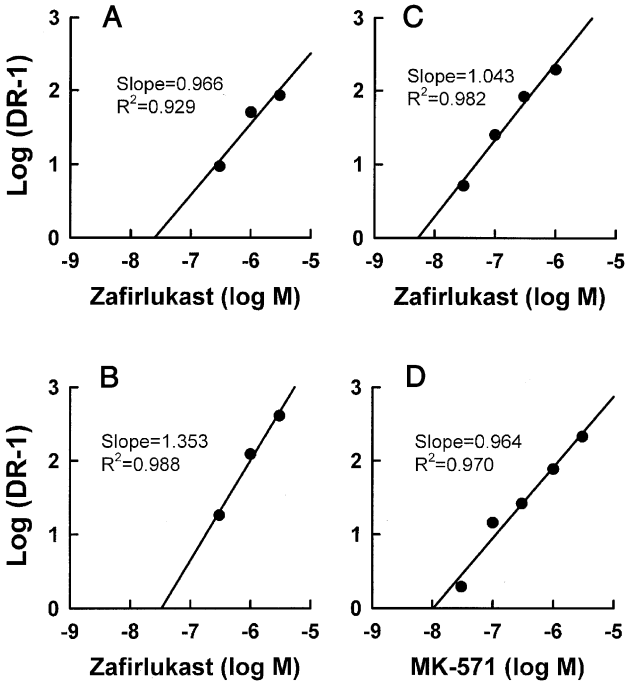


Fig. 3. Schild plot analysis of the $CysLT_1$ receptor antagonist zafirlukast on the contractions induced by leukotriene $C_4$ (panel A), $D_4$ (panel B) and $E_4$ (panel C). Panel D show the Schild plot analysis of the $CysLT_1$ receptor antagonist MK-571 on the contractions induced by leukotriene $E_4$. All experiments were performed in the presence of L-serine borate (45 mM) and L-cysteine (5 mM). Zafirlukast, MK-571, L-serine borate and L-cysteine were applied 30 min before the addition of leukotrienes. Each point represents the mean of log of (dose ratio − 1).

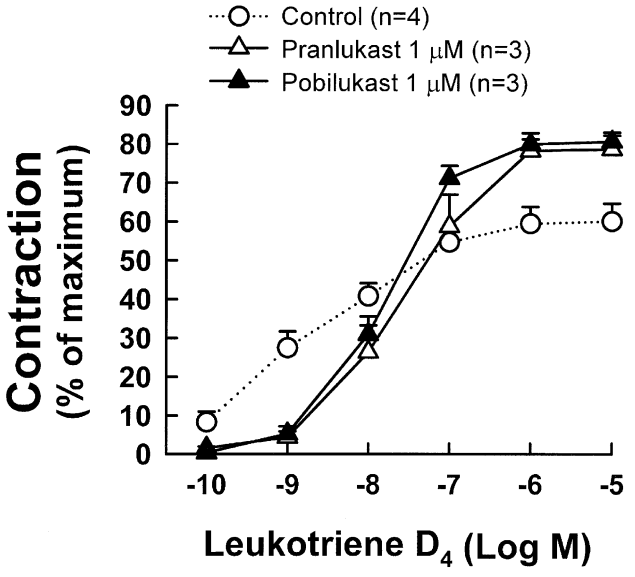


Fig. 4. Effects of the $CysLT_1$ receptor antagonists, pranlukast and pobilukast on the contractions induced by leukotriene $D_4$ in the guinea pig lung parenchyma in the presence of L-serine borate (45 mM) and L-cysteine (5 mM). Pranlukast, pobilukast, L-serine borate and L-cysteine were applied 30 min before the addition of leukotrienes. Each point represents the mean ± S.E.M, with the number of experiments indicated in parentheses.

### *3.3. Effects of cyclooxygenase inhibition and nitric oxide synthase inhibition on cysteinyl-leukotriene-induced contractions*

In the presence of metabolic inhibitors for cysteinyl-leukotrienes, the cyclooxygenase inhibitor indomethacin (10 μM) significantly decreased the p$D_2$ and slightly but significantly potentiated the $E_{max}$ for leukotriene $D_4$ (Fig. 5A and Table 4). The nitric oxide synthase inhibitor L-NOARG (100 μM) further enhanced the $E_{max}$ without changing the p$D_2$ for leukotriene $D_4$ in the presence of indomethacin (Fig. 5A and Table 4). Neither the $E_{max}$ nor the p$D_2$ for leukotriene $D_4$ in the presence of the combination of indomethacin and L-NOARG was significantly different from those obtained in

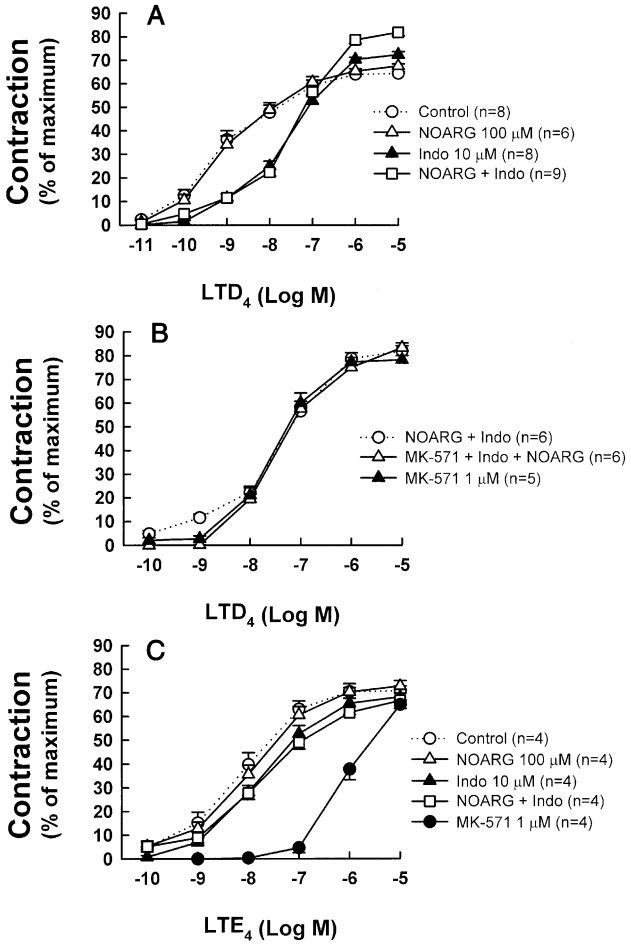


Fig. 5. Effects of indomethacin (10 μM, Indo) alone and the combination of L-NOARG (100 μM, NOARG) with indomethacin on the contraction induced by leukotriene $D_4$ in the guinea pig lung parenchyma (panel A). Panel B shows the effects of MK-571 (1 μM) in the absence or presence of the combination of L-NOARG and indomethacin. Panel C shows the effects of MK-571, indomethacin alone and the combination of L-NOARG (100 μM) and indomethacin on contraction induced by leukotriene $E_4$. MK-571, indomethacin, L-NOARG, L-serine borate (45 mM) and L-cysteine (5 mM) were applied 30 min before the addition of leukotrienes. Each point represents the mean ± S.E.M, with the number of experiments indicated in parentheses.

Table 4
Effects of indomethacin (Indo, 10 μM), L-NOARG (NOA, 100 μM) and MK-571 on the $E_{max}$ and p$D_2$ for the contractile effects of leukotriene $D_4$ in the guinea pig lung parenchyma

| | *n* | $E_{max}$ | p$D_2$ |
|---|---|---|---|
| Control | 8 | 64.7 ± 0.95 | 8.88 ± 0.17 |
| Indo | 8 | 72.4 ± 1.30[a] | 7.37 ± 0.06[a] |
| NOA + Indo | 8 | 81.9 ± 1.48[a,b] | 7.24 ± 0.02[a] |
| MK-571 + NOA + Indo | 8 | 83.3 ± 1.98[a,b] | 7.21 ± 0.03[a] |
| MK-571 | 8 | 78.2 ± 1.77[a,b] | 7.31 ± 0.08[a] |

MK-571, indomethacin, L-NOARG, L-serine borate (45 mM) and L-cysteine (5 mM) were applied 30 min before the addition of leukotriene $D_4$. Each data represents the mean ± S.E.M. of *n* experiments.
[a] $P < 0.05$ vs. Control.
[b] $P < 0.05$ vs. Indo (Tukey test).

the presence of MK-571 (Fig. 5 and Table 4). The combination of these three treatments did not further alter either the $E_{max}$ or the p$D_2$ (Fig. 5 and Table 4).

Indomethacin significantly decreased the p$D_2$ for leukotriene $E_4$ without changing the $E_{max}$ (Fig. 5C). L-NOARG (100 μM) did not further change the $E_{max}$ and p$D_2$ in the presence of indomethacin (Fig. 5C). The p$D_2$ for leukotriene $E_4$ in the presence of indomethacin and L-NOARG were significantly weaker than those in the presence of MK-571 (1 μM, Fig. 5C).

## 4. Discussion

Using different structures of $CysLT_1$ receptor antagonists, it was demonstrated that $CysLT_1$ receptor antagonists inhibited the contractions induced by leukotriene $C_4$, $D_4$ and $E_4$ in the guinea pig lung parenchyma. However, the contractions to leukotriene $E_4$ were inhibited more effectively than those induced by either leukotriene $C_4$ or $D_4$, suggesting a differential receptor activation by the individual cysteinyl-leukotrienes.

$CysLT_1$ receptor antagonists inhibited the contraction to leukotriene $E_4$ in a competitive manner, indicating that leukotriene $E_4$ solely activated one CysLT receptor in the guinea pig lung parenchyma. The p$A_2$ for zafirlukast and MK-571 obtained in this study (8.3 and 8.0, respectively) were similar to their binding affinities to the human cloned $CysLT_1$ receptor ($IC_{50}$ of 2 and 10 nM, respectively)(Lynch et al., 1999). Therefore, the receptor mediating the contraction to leukotriene $E_4$ in the present study is most likely a $CysLT_1$ receptor.

The less effective antagonism of the contractions to either leukotriene $C_4$ or $D_4$ suggests that, in addition to the $CysLT_1$ receptor, also another CysLT receptor is involved in the contractions to these leukotrienes. These results are similar to results from human and porcine pulmonary arteries, which have been reported to contain a CysLT receptor with similar selectivity to cysteinyl-leukotrienes, i.e., stimulated by leukotriene $C_4$ and $D_4$ but not by leukotriene $E_4$ (Bäck et al., 2000a,b; Schellenberg and Foster, 1984).

The cyclooxygenase inhibitor indomethacin induced a rightward shift and enhanced the $E_{max}$ of the concentration–response curves for leukotriene $D_4$, suggesting a modulation by both contractile and relaxant prostanoids. It has previously been reported that in the presence of a cyclooxygenase inhibitor, $CysLT_1$ receptor antagonists either not at all (Weichman et al., 1982) or slightly (Norman et al., 1987) inhibit the contraction to leukotriene $D_4$ in the guinea pig lung parenchyma. The present study extends these findings by showing an identical inhibition of p$D_2$ by indomethacin and $CysLT_1$ receptor antagonists. Together, these results suggest that release of contractile prostanoids, such as thromboxane $A_2$ (Dahlén et al., 1983), may be linked to a $CysLT_1$ receptor in the guinea pig lung parenchyma and that the rightward shift of the concentration–response curves for leukotriene $D_4$ by MK-571 is mainly mediated by inhibition of $CysLT_1$ receptor-mediated prostanoid release.

The $E_{max}$ for leukotriene $C_4$ and $D_4$ were enhanced by pretreatment with MK-571, suggesting that MK-571 inhibited also the release of relaxant prostanoids. However, the enhancement of the $E_{max}$ by pretreatment with indomethacin was less than that obtained with MK-571, and only the combination of indomethacin and L-NOARG could completely mimic the effect of the $CysLT_1$ receptor antagonist. In addition, MK-571 did not further potentiate the $E_{max}$ in the presence of indomethacin and L-NOARG. These latter observations suggest that release of nitric oxide may also be involved in the enhancement of the $E_{max}$ for leukotriene $D_4$ observed after treatment with $CysLT_1$ receptor antagonists in the guinea pig lung parenchyma. CysLT receptors have previously been proposed to be linked to nitric oxide production in the guinea pig pulmonary artery (Sakuma et al., 1987) and the human pulmonary vein (Ortiz et al., 1995).

In addition to MK-571, also two other structurally unrelated $CysLT_1$ receptor antagonists, pranlukast and pobilukast, enhanced the $E_{max}$ for leukotriene $D_4$. In contrast, zafirlukast had a different pattern of inhibition on the contraction to either leukotriene $C_4$ or $D_4$, since this compound did not alter the $E_{max}$ of the contraction to these leukotrienes. Interestingly, similar differences by $CysLT_1$ receptor antagonists have been reported in human airway smooth muscle (Panettieri et al., 1998) where pranlukast and pobilukast, but not zafirlukast, inhibited the potentiation of epidermal growth factor-induced proliferation by leukotriene $D_4$. In addition, Ravasi et al. (2000) also reported that pranlukast and pobilukast competed for the binding of either [$^3$H]leukotriene $C_4$ or [$^3$H]leukotriene $D_4$, whereas zafirlukast competed only for the binding of [$^3$H]leukotriene $D_4$ in human lung parenchyma. Taken together, these results suggest that different $CysLT_1$ receptor antagonists may recognise different CysLT receptors. It can however not be excluded that there are differences in specificity between the antagonists. For example, MK-571 was recently reported to interact with the receptor for lipoxin $A_4$ (Gronert et al., 2001).

Previous reports on the effects of $CysLT_1$ receptor antagonists on leukotriene $D_4$-induced contractions in the guinea pig lung parenchyma are heterogeneous. For instance, the $pK_B$ or $pA_2$ of ICI-198,615 for leukotriene $D_4$ have been reported to be 9.5 (Snyder et al., 1987), 6.3 (Norman et al., 1987), 5.7 (Tudhope et al., 1994) and 7.2 (Wikström Jonsson et al., 1998). One probable reason for these differences is the involvement of the metabolic conversion of cysteinyl-leukotrienes in the guinea pig lung parenchyma (Dahlén et al., 1983). Since leukotriene $E_4$ and leukotriene $D_4$ had different preferences for CysLT receptors in the present study, the metabolic conversion may affect the pharmacological results of CysLT receptor antagonists in this preparation. In addition, the involvement of prostanoids is another probable reason for the heterogeneous results, since the cyclooxygenase inhibitor caused profound changes of the potencies of the $CysLT_1$ receptor antagonists in the guinea pig lung parenchyma (Norman et al., 1987; present study). Thirdly, the concentrations of $CysLT_1$ receptor antagonists used by different investigators may also contribute to the heterogeneous pharmacological results, since the effects of $CysLT_1$ receptor antagonists on leukotriene $D_4$-induced contractions were not competitive in the present study. For example, Tudhope et al. (1994) used only a high concentration (10 μM) of ICI-198,615.

In conclusion, the results of the present study using several concentrations of different $CysLT_1$ receptor antagonists, suggest that leukotriene $E_4$ solely activates a $CysLT_1$ receptor, whereas leukotriene $C_4$ and $D_4$ activate also another CysLT receptor in the guinea pig lung parenchyma. Since the contractions to leukotriene $C_4$ and $D_4$ in the guinea pig lung parenchyma have been reported not to involve the activation of a $CysLT_2$ receptor (Tudhope et al., 1994; Wikström Jonsson et al., 1998), leukotriene $C_4$ and $D_4$ but not leukotriene $E_4$ may activate a purported new CysLT receptor. The present observations also suggest that the $CysLT_1$ receptor antagonist-resistant part of the contraction to leukotriene $D_4$ can be focused by the combination of indomethacin and L-NOARG. This preparation in the presence of a cyclooxygenase inhibitor and a nitric oxide synthase inhibitor may be a useful tool in search of molecular structures of further CysLT receptors.

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