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# The effect of indomethacin on the contractile response of the guinea-pig lung parenchymal strip to leukotrienes $B_4$, $C_4$, $D_4$ and $E_4$

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**1** Indomethacin (1 $\mu g\,ml^{-1}$) almost totally inhibited the dose-dependent contractile response of isolated lung parenchymal strips of the guinea-pig (GPLS) to leukotriene $B_4$ ($LTB_4$) over the concentration range 0.18–18 nM.

**2** $LTC_4$ (0.63 pM–63 nM)-induced contractions of GPLS were not significantly inhibited by indomethacin (1.0 and 10.0 $\mu g\,ml^{-1}$) except when the highest $LTC_4$ concentration (63 nM) was tested in the presence of indomethacin (10 $\mu g\,ml^{-1}$).

**3** $LTD_4$ (1.3 fM–13 nM)-induced contractions of GPLS were not significantly inhibited by indomethacin (0.1–10 $\mu g\,ml^{-1}$) except for contractions induced by concentrations of $LTD_4$ greater than 0.13 nM and 13 nM. Indomethacin 1 $\mu g\,ml^{-1}$ and 10 $\mu g\,ml^{-1}$ inhibited the contractile response to 13 nM $LTD_4$ by 37 and 16% respectively.

**4** $LTE_4$ (2.3 fM–23 nM)-induced contractions of GPLS were not significantly inhibited by indomethacin (0.1–10 $\mu g\,ml^{-1}$). Contraction due to $LTE_4$ 23 pM was significantly potentiated by indomethacin (1 $\mu g\,ml^{-1}$).

**5** Clotrimazole (10 $\mu M$) significantly inhibited $LTD_4$-induced contractions of GPLS at concentrations greater than 13 pM but had no significant effect on $LTC_4$-induced contractions.

**6** Cyclo-oxygenase products, probably principally thromboxane $A_2$, are important secondary mediators of $LTB_4$-induced contractions of GPLS but make little or no contribution to contractions of GPLS induced by $LTC_4$, $LTD_4$, and $LTE_4$, except at higher concentrations of $LTD_4$ and possibly $LTC_4$. Certain concentrations of $LTE_4$ may generate bronchodilator $PGE_2$ in GPLS.

## Introduction

Slow reacting substance (Kellaway & Trethewie, 1940), an activity generated during immediate type hypersensitivity reactions and designated slow reacting substance of anaphylaxis (SRS-A) (Brocklehurst, 1960), is now known to be composed of leukotrienes (LT) $C_4$, $D_4$ and $E_4$ (Murphy, Hammarström & Sammuelsson, 1979; Morris, Taylor, Piper & Tippins, 1980; Lewis, Drazen, Austen, Clark & Corey, 1980b; Lewis, Austen, Drazen, Clark, Marfat & Corey, 1980a). $LTC_4$ and $LTD_4$ are potent constrictors of guinea-pig bronchial smooth muscle *in vivo* and *in vitro* (Drazen, Austen, Lewis, Clark, Goto, Marfat & Corey, 1980). Injection of SRS-A (Mathé, Strandberg & Yen, 1977; Engineer, Morris, Piper & Sirois, 1978), $LTC_4$ or $LTD_4$ (Piper & Samhoun, 1981; Omini, Folco, Vigano, Rossoni, Brunelli & Berti, 1981) into isolated perfused lungs of guinea-pig causes the release of prostaglandins and thromboxanes which can be prevented by pretreatment with the cyclo-oxygenase inhibitor, indomethacin (Piper & Samhoun, 1981). Two observations suggest that these released cyclo-oxygenase products may be important secondary mediators of $LTC_4$- and $LTD_4$-induced bronchial smooth muscle contractions in the guinea-pig; firstly, intravenous infusion of $LTC_4$ or $LTD_4$ into anaesthetized guinea-pigs induced bronchoconstriction which was abolished by pretreatment with aspirin or indomethacin (Omini *et al.*, 1981; Schiantarelli, Bongrani & Folco, 1981; Vargäftig, Lefort & Murphy, 1981); secondly pretreatment with indomethacin (1 $\mu g\,ml^{-1}$) substantially inhibited the guinea-pig lung parenchymal strip (GPLS) contractile response due to $LTC_4$ (10–100 pM) and

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$LTD_4$ (1 pM) *in vitro* (Piper & Samhoun, 1981; Zijlstra, Adolfs, Vincent & Bonta, 1983), and pretreatment with aspirin (10.6 $\mu g\,ml^{-1}$) diminished the GPLS contractile response due to $LTC_4$ (10 pM) by more than 50% (Vargäftig *et al.*, 1981).

The availability of adequate quantities of synthetic $LTB_4$, $LTC_4$, $LTD_4$ and $LTE_4$ has allowed us to study the concentration-effect curves for these agonists over a wider range of concentrations than previously reported in the presence and absence of different concentrations of indomethacin and also of the thromboxane synthesis inhibitor, clotrimazole.

## Methods

Male Hartley strain guinea-pigs, 300–400 g body weight, were killed by cervical dislocation and exsanguination. The thorax was opened, and the heart, lungs and trachea were removed en bloc. Strips of sub-pleural pulmonary parenchyma (approximately 1.5 mm square and 20 mm long) were cut from pulmonary lobes and suspended in a bath of Tyrode solution (Tyrode, 1910). The solution was continually gassed with 95% $O_2$ and 5% $CO_2$. The lower end of the parenchymal strip was fixed to a support in the bath, while the upper end was attached by a thread to a force transducer (Grass Instrument FTO13C) under an initial force of 1 g. After 60 min to allow relaxation of the tissues, a cumulative histamine concentration-effect curve was obtained by adding histamine to the organ bath to establish log increments of bath histamine concentrations over the range of 10 nM–100 $\mu$M (Drazen & Schneider, 1978). Thereafter the tissues were washed at 15 min intervals for 1 h before leukotriene concentration-effect curves were determined. Leukotrienes were added to the bath to establish log increments of leukotriene concentrations in the bath for a cumulative concentration-effect curve within the range of 1.3 fM–63 nM (Drazen *et al.*, 1980). After each increment, the tissue response was observed for 3–5 min until a plateau was achieved. When the effect of indomethacin was studied, indomethacin was present in the perfusing Tyrode solution in concentrations of 0.1, 1.0 and 10 $\mu g\,ml^{-1}$ from the beginning of each experiment.

### *Drugs*

The drugs used in this study were histamine diphosphate, clotrimazole and indomethacin (Sigma Chemical Co., St. Louis, MO). Synthetic leukotrienes were synthesized according to published methods (Lewis *et al.*, 1980a; Corey, Clark, Goto, Marfat, Mioskowski, Samuelsson & Hammarström, 1980a; Corey, Clark, Marfat & Goto, 1980b; Corey, Marfat, Goto & Brion, 1980c); purity and concentration were established by absorbance and retention time on reverse phase high performance liquid chromatography (A269 for $LTB_4$; A280 for other leukotrienes) by use of a standard buffer with isocratic elution (Lewis *et al.*, 1980a). Leukotrienes were stored at −80°C in 20% ethanol and phosphate buffer at pH 6.8 under argon before use. Drugs were freshly prepared on the day of use and, except as noted, diluted in Tyrode buffer to an appropriate concentration immediately before use. Indomethacin was prepared in absolute ethanol before dilution in Tyrode buffer. Bath concentrations of ethanol did not exceed 0.1% and this concentration was without effect on the baseline of the tissues or leukotriene-induced contractions. Indomethacin 0.1–10 $\mu g\,ml^{-1}$ also did not affect tissue baselines. The effective concentrations of agonist required for inducing responses to 25% and 50% of those achieved by 100 nM histamine were computed from concentration-effect curves and termed the EC25 and EC50 respectively.

Differences between means were tested according to Student's $t$ test, and a $P$ value of $< 0.05$ was considered significant.

## Results

### *Effect of indomethacin on GPLS contractile responses to leukotrienes*

$LTB_4$ produced a concentration-dependent contraction of GPLS ($EC_{25}$ 10 nM) which was significantly inhibited by indomethacin at 1 $\mu g\,ml^{-1}$ over the concentration range 0.18–18 nM (Figure 1).

The concentration-dependent contraction of GPLS elicited by $LTC_4$ ($EC_{50}$ 7.2 nM) was not sig-

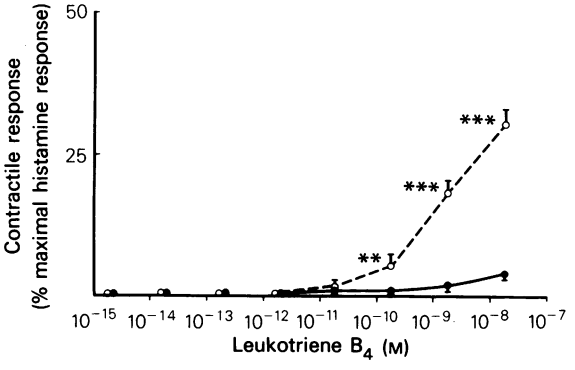


**Figure 1** Contractile response of guinea-pig lung parenchymal strip to leukotriene $B_4$, (expressed as % of response to $10^{-7}$M histamine) in the presence (●) and absence (○) of indomethacin (1 $\mu g\,ml^{-1}$). $n = 10$ for each point. Probability values: ** $P < 0.01$; *** $P < 0.001$.

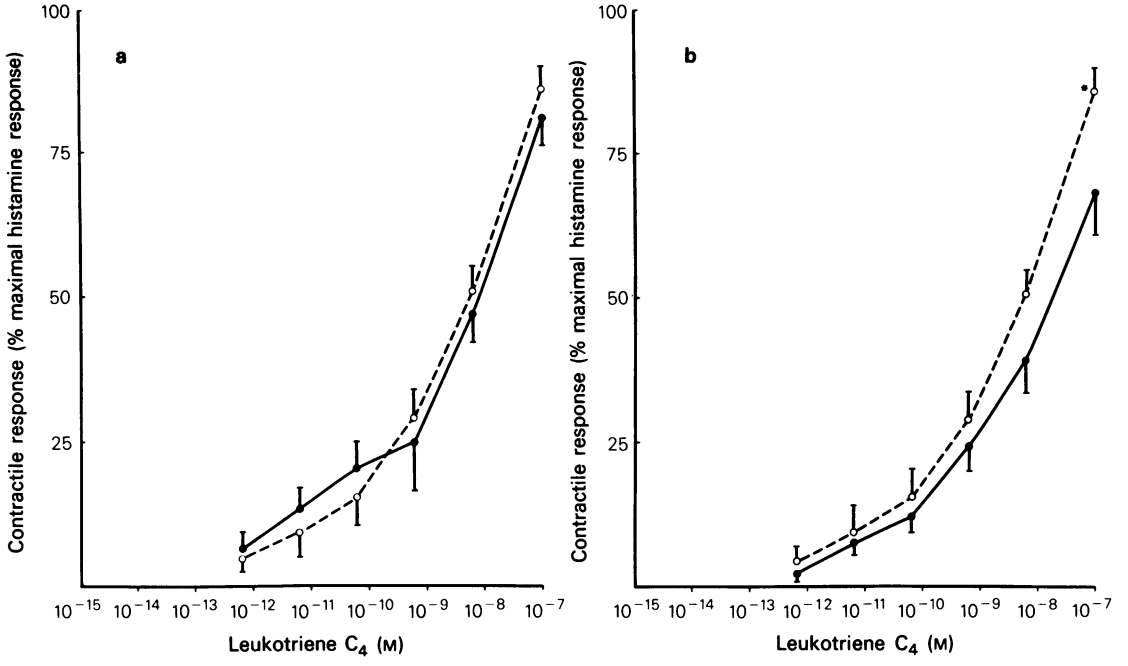


**Figure 2** Contractile response of guinea-pig lung parenchymal strips to leukotriene $C_4$ in the presence (●) ($n = 10$) and absence (○) ($n = 11$) of indomethacin (1 $\mu g\,ml^{-1}$ in (a) and 10 $\mu g\,ml^{-1}$ in (b)). Probability value: $^*P < 0.05$.

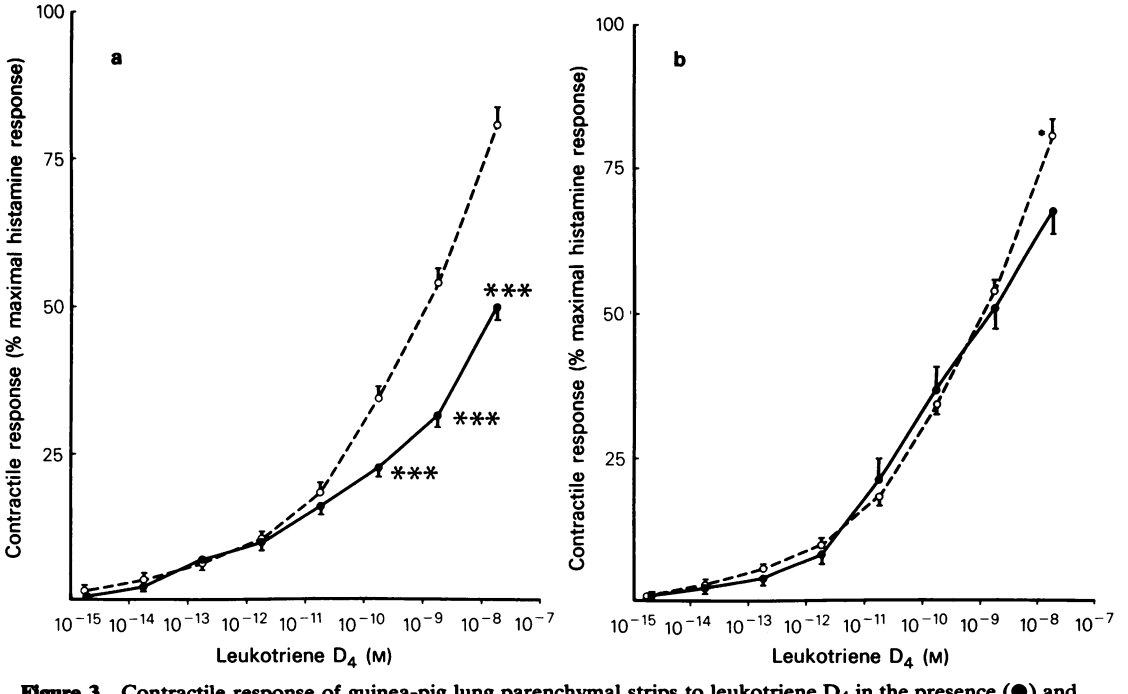


**Figure 3** Contractile response of guinea-pig lung parenchymal strips to leukotriene $D_4$ in the presence (●) and absence (○) of indomethacin (1 $\mu g\,ml^{-1}$ in (a) and 10 $\mu g\,ml^{-1}$ in (b)). In (a) $n = 21$ in absence of indomethacin; $n = 18$ in presence of indomethacin; in (b) $n = 21$ in absence of indomethacin; $n = 6$ in presence of indomethacin. Probability values: $^*P < 0.05$; $^{***}P < 0.001$.

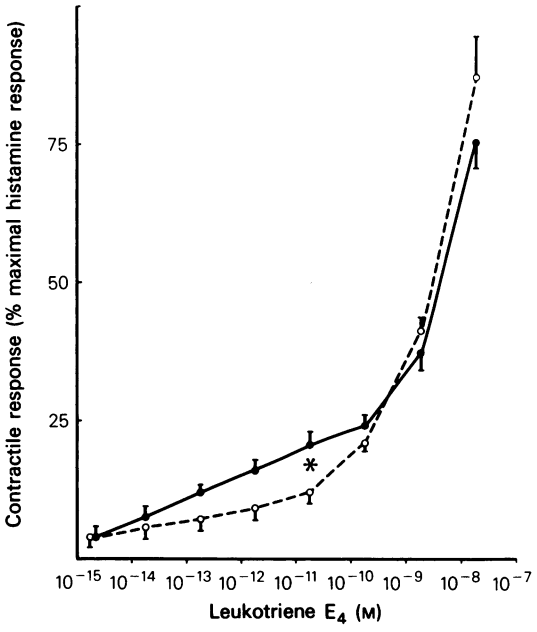


**Figure 4** Contractile response of guinea-pig lung parenchymal strips to leukotriene $E_4$ in the presence (●, $n=6$) and absence (○, $n=6$) of indomethacin ($1\,\mu g\,ml^{-1}$). Probability value: $^*P<0.05$.

nificantly affected by indomethacin in bath concentrations of 1.0 and $10\,\mu g\,ml^{-1}$ (Figure 2a, b) except for the minimally significant inhibition of contraction due to $LTC_4$ 63 nM by indomethacin at $10\,\mu g\,ml^{-1}$ (Figure 2b).

The $EC_{50}$ of $LTD_4$-induced contraction of GPLS was 1.3 nM and the concentration-effect curve was not significantly different in the presence of indomethacin at 0.1 and $10\,\mu g\,ml^{-1}$ except for 16% inhibition at 13 nM by indomethacin at $10\,\mu g\,ml^{-1}$ (Fig 3b). However, indomethacin at $1\,\mu g\,ml^{-1}$ produced significant ($P<0.001$) inhibition of the contractile response over the concentration range (0.13–13 nM) for $LTD_4$ (Figure 3a) achieving 37% inhibition of the contraction induced by $LTD_4$ (13 nM).

$LTE_4$ produced concentration-dependent contraction of GPLS ($EC_{50}$ 2.3 nM) which was not significantly inhibited by indomethacin at 0.1, 1.0 or $10\,\mu g\,ml^{-1}$ (Figure 4a). When administered at $1\,\mu g\,ml^{-1}$, indomethacin significantly potentiated the contractile response to $LTE_4$ 23 pM (Figure 4).

### *Effect of clotrimazole on contractile responses of GPLS due to leukotrienes $C_4$ and $D_4$*

Clotrimazole ($10\,\mu M$) produced significant inhibition

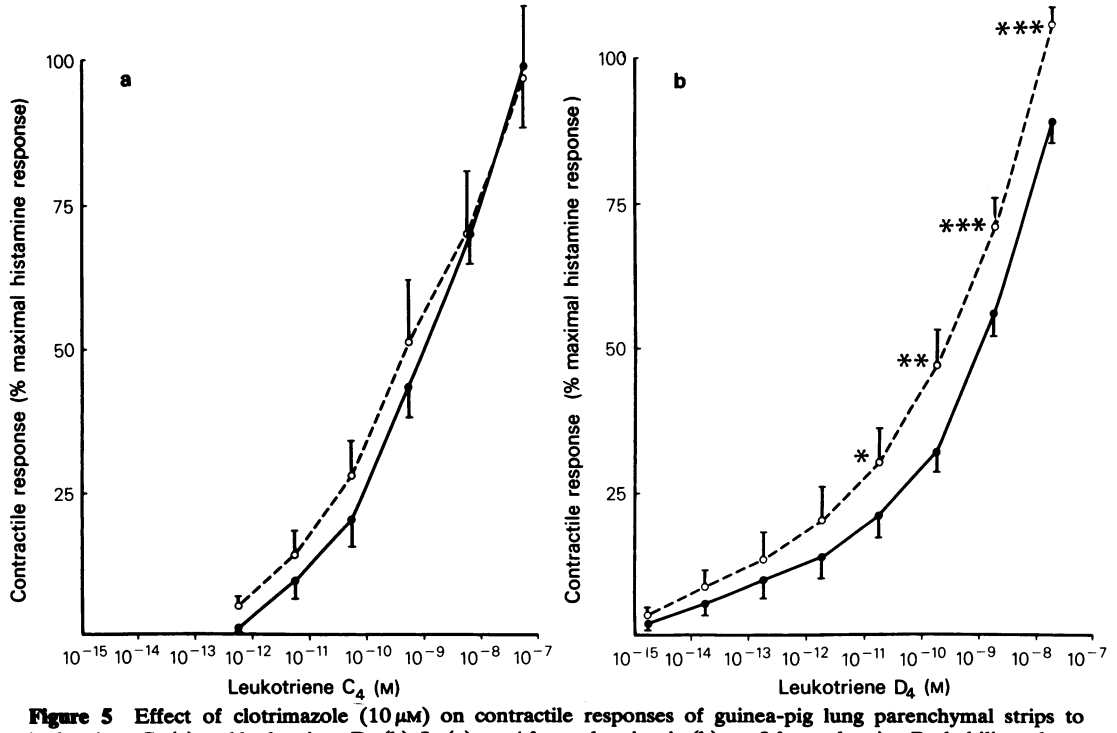


**Figure 5** Effect of clotrimazole ($10\,\mu M$) on contractile responses of guinea-pig lung parenchymal strips to leukotriene $C_4$ (a) and leukotriene $D_4$ (b). In (a) $n=4$ for each point; in (b) $n=8$ for each point. Probability values: $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

of GPLS contractile response to $LTD_4$ over the concentration-range 13 pM–13 nM (Figure 5b) but had no effect on the contractile response to $LTC_4$ (Figure 5a).

## Discussion

The inhibition by indomethacin at 1 $\mu g\,ml^{-1}$ of the $LTB_4$-induced contraction of GPLS observed in this study confirms previous findings (Sirois, Borgeat, Jeanson, Roy & Girard, 1980) and contrasts with the relative failure of this agent to modify the response of the same preparation to the sulphidopeptide leukotrienes. The same concentration of indomethacin (1 $\mu g/ml$) has no significant effect on the contractile response to GPLS due to $LTC_4$ (0.63 pM–63 nM), low concentrations of $LTD_4$ (1.3 fM–13 pM) or most concentrations of $LTE_4$. However, this concentration of indomethacin significantly inhibited the contractile response due to higher concentrations of $LTD_4$ (0.13–13 nM) and significantly potentiated the contractile response to one concentration (23 pM) of $LTE_4$. These findings suggest that characteristic profiles of cyclo-oxygenase products generated by contractions of GPLS may result from the action of different concentrations and classes of the three sulphidopeptide leukotrienes. The apparent effects include the preferential release of a bronchoconstrictor product by $LTD_4$, the possible release of a bronchodilator product by $LTE_4$ and the release of no secondary products or of equal amounts of bronchoconstrictor and bronchodilator activities by $LTC_4$. Our findings differ from those reported by others (Weichman, Muccitelli, Osborn, Holden, Gleason & Wasserman, 1982) who found that indomethacin (1 $\mu M$) or meclofenamic acid (1 $\mu M$) produced approximately parallel shifts in the concentration-effect curves for $LTC_4$, $D_4$ and $E_4$. However, these investigators used only one concentration of each inhibitor and their leukotrienes were diastereomeric mixtures which resulted in $EC_{50}$s about 1–8 times greater than those found in the present study. No other published study of the effect of indomethacin on the GPLS contractile responses to the range of concentrations of pure synthetic $LTB_4$, $LTC_4$, $LTD_4$ and $LTE_4$ is available for comparison.

Clotrimazole (10 $\mu M$), a thromboxane synthetase inhibitor (Gordon, Nouri & Thomas, 1981), significantly inhibited the contractile response of GPLS to $LTD_4$, thus implicating thromboxane $A_2$ as the principal bronchoconstrictor activity released by $LTD_4$. Significant inhibition by clotrimazole of $LTD_4$-induced contractions of GPLS extended over a wider range of concentrations (13 pM–13 nM) than the inhibition observed with indomethacin at 1 $\mu g$ ml. This difference could be explained by the generation of a significant bronchodilator cyclo-oxygenase activity by $LTD_4$ which was inhibited by indomethacin but not by clotrimazole.

Indomethacin at 0.1 $\mu g\,ml^{-1}$ was without effect on all sulphidopeptide LT-induced contractile responses of GPLS. The highest concentration of indomethacin (10 $\mu g\,ml^{-1}$) did inhibit contractile response due to the highest concentrations of $LTC_4$ (63 nM) and $LTD_4$ (13 nM) but failed to inhibit the lower concentrations of $LTD_4$ which had been suppressed by 1 $\mu g\,ml^{-1}$. The discrepancy between the effects of indomethacin at 1 and 10 $\mu g\,ml^{-1}$ on the contractile responses to $LTD_4$ could result from differential effects of the two concentrations of indomethacin on the quantity and type of the cyclo-oxygenase products generated. Similar bell-shaped concentration-effect curves for indomethacin have been reported by others for related systems in guinea-pig lung (Engineer *et al.*, 1978; Adcock & Garland, 1980).

Our data therefore suggest that the profile of cyclo-oxygenase products released by each of the sulphidopeptide leukotrienes may differ for each leukotriene and possibly for different concentrations of each leukotriene. The consequences of pretreatment with indomethacin will depend on the concentration of indomethacin employed, as well as on its effect on the particular profile of cyclo-oxygenase products resulting from a particular concentration of each leukotriene.

The findings that indomethacin at 1 $\mu g\,ml^{-1}$ and clotrimazole at 10 $\mu M$ failed significantly to inhibit $LTC_4$-induced contractions of GPLS but that both inhibitors significantly affected $LTD_4$-induced contractions of GPLS does not support the argument advanced by others (Morris, Taylor, Jones, Piper, Samhoun & Tippins, 1982), that bioconversion of $LTC_4$ to $LTD_4$ is necessary for biological activity of $LTC_4$.

$LTC_4$ and $LTD_4$ administered intravenously to the guinea-pig cause bronchoconstriction with an early indomethacin-inhibitable component with preferential activity on small airways and a later slow-reacting component which is potentiated by indomethacin (Leitch, Austen, Corey & Drazen, 1982). Thromboxane $A_2$ is the most likely candidate for the secondarily mediated bronchoconstriction seen in the first minute following infusion of leukotrienes. Evidence supporting this hypothesis is that thromboxanes are present in the plasma after intravenous infusion of leukotrienes to the guinea-pig (Omini *et al.*, 1981; Schiantarelli *et al.*, 1981); thromboxane $A_2$ has a short biological half-life (Hamberg, Svensson & Samuelsson, 1975a; Hamberg, Hedqvist, Strandberg, Svensson & Samuelsson, 1975b), a preferential

action on small airways (Schneider & Drazen, 1980) and, finally, the selective thromboxane synthetase inhibitor, OKY 1581, partially inhibits airway constriction resulting from intravenous sulphidopeptide leukotrienes (Ueno, Tanaka, Hirose, Shishido & Katori, 1983).

However, when $LTC_4$ or $LTD_4$ are applied locally to the lungs of guinea-pigs *in vivo* by aerosol, cyclo-oxygenase inhibitors have no inhibitory effect on the resulting bronchoconstriction but rather significantly potentiate the bronchoconstrictor response (Weichman, Muccitelli, Osborn, Holden, Gleason & Wasserman, 1982; Hamel, Masson, Ford-Hutchinson, Jones, Brunet & Piechuta, 1982; Leitch, Corey, Austen & Drazen, 1983). This effect may be attributed to inhibition of generation of $PGE_2$ which is released in response to contractile agonists, including leukotrienes (Adcock & Garland, 1980; Brink, Duncan & Douglas, 1981; Krell, Osborn, Vickery, Falcone, O'Donnell, Gleason, Kinzig & Bryan, 1981) and exerts a bronchodilator regulatory effect (Orehek, Douglas, Lewis & Bouhuys, 1973). The discrepancy between the effects of pretreatment with indomethacin on the pulmonary response to intravenous leukotrienes and those given by aerosol in the guinea-pig suggests that thromboxanes are not generated locally in significant quantities in the lung in response to local leukotriene administration.

Our *in vitro* findings also suggest that the principal component of sulphidopeptide leukotriene-induced contraction of GPLS is primary. Previous reports of a substantial contribution of thromboxane $A_2$ to the $LTC_4$- and $LTD_4$-induced contractions of GPLS, at leukotriene concentrations of less than 100 pM (Piper & Samhoun, 1981; Zijlstra *et al.*, 1982) are based upon the superfusion cascade system where the leukotriene-induced contraction was shown to be mediated by thromboxane $A_2$, possibly released as a consequence of tissue agitation (Orehek *et al.*, 1973). An entirely primary contractile effect of $LTC_4$ was also observed by others (Dahlén, Hedqvist, Granström, Lindgren & Petroni, 1983), in a conventional organ bath such as was used in the present study.

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