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The contractile activities of lipoxin A_4 and lipoxin B_4 for guinea-pig airway tissues

Crawford A.J. Jacques, Bernd W. Spur, Attilio E.G. Crea, [1]Tak H. Lee

Department of Medicine, United Medical and Dental Schools, Guy's Hospital, London SE1 9RT

1 The isometric contractile activities of lipoxin A_4 (LxA_4) and lipoxin B_4 (LxB_4) were evaluated on guinea-pig lung tissue over the concentration range, 10^{-8} to 10^{-5} M.

2 LxA_4 contracted guinea-pig lung parenchymal strips; the concentration eliciting 50% maximum histamine response was 3×10^{-6} M. LxA_4 did not contract tracheal spirals.

3 The LxA_4 dose-response curve was parallel to that of leukotriene D_4 (LTD_4) with LxA_4 being approximately 10,000 fold less potent than LTD_4.

4 The time course of the contraction elicited by LxA_4 was similar to that of LTD_4 and it was slow in onset and did not plateau for 20 min.

5 Pre-incubation of parenchymal strips with leukotriene receptor antagonists at a concentration of 1×10^{-6} M to 3×10^{-5} M FPL 55712 or 3×10^{-5} M L 649923 inhibited LxA_4 activity.

6 Pre-incubation of tissues with 1×10^{-5} M L 651392, a 5-lipoxygenase inhibitor, or 1×10^{-5} M indomethacin, a cyclo-oxygenase inhibitor, did not affect the contractile activity of LxA_4.

7 LxB_4 did not constrict parenchymal strips or tracheal spirals.

Introduction

Non-esterified arachidonic acid may be metabolized by cyclo-oxygenase or lipoxygenase pathways (Samuelsson, 1983). Three major lipoxygenase pathways have been identified in mammalian tissues. These include the 5-, 12- and 15-lipoxygenases which transform arachidonic acid into a number of biologically active metabolites. The 5-lipoxygenase pathway converts arachidonic acid to 5S-hydroperoxy-6,8,11,14 eicosatetraenoic acid which may be further transformed into leukotrienes (Samuelsson, 1983). Leukotriene B_4 (LTB_4) is a potent chemotactic agent (Ford-Hutchinson *et al.*, 1980; Palmer *et al.*, 1980; Nagy *et al.*, 1982; Soter *et al.*, 1983; Camp *et al.*, 1983) and is a complete secretagogue in human neutrophils (PMN) (Showell *et al.*, 1982). Leukotriene C_4, D_4 and E_4 (LTC_4, LTD_4 and LTE_4) comprise the activity previously known as slow reacting substance of anaphylaxis (SRS-A) (Murphy *et al.*, 1979; Orning *et al.*, 1980; Morris *et al.*, 1980; Lewis *et al.*, 1980; Piper, 1984). These sulphidopeptide leukotrienes increase vascular permeability and contract non-vascular smooth muscle. The leukotrienes all contain a conjugated triene structure (Figure 1) and may serve as important mediators in both immediate hypersensitivity reactions and in inflammatory processes (Samuelsson, 1983).

Recently a novel series of arachidonic acid-derived metabolites have been described as being produced through the interactions of the 5- and 15-lipoxygenase pathways (Serhan *et al.*, 1984a,b). The distinguishing feature of these metabolites is the presence of a trihydroxy tetraene structure (Figure 1). Since these metabolites arise from the interaction of multiple distinct lipoxygenase pathways, the name lipoxins has been designated for this series of compounds. Two biologically active lipoxins have been identified, lipoxin A_4 (LxA_4) and lipoxin B_4 (LxB_4). LxA_4 is 5,6,15-trihydroxy-7,9,11,13-eicosatetraenoic acid (Serhan *et al.*, 1986a) and LxB_4 is 5,14,15-trihydroxy-6,8,10,12-eicosatetraenoic acid (Serhan *et al.*, 1986b).

Information on the biological activities of these compounds is very limited. The available data indicate that LxA_4, but not LxB_4 contracts guinea-pig lung parenchymal strips (Serhan *et al.*, 1986a), is chemokinetic for human neutrophils (Spur *et al.*, 1988) and induces superoxide anion generation and elastase release from human neutrophils (Serhan *et al.*, 1985). Human natural killer cells exposed to either LxA_4 or LxB_4 are unable to provoke target cell lysis (Ramstedt *et al.*, 1985). The relative lack of data on

[1] Author for correspondence.

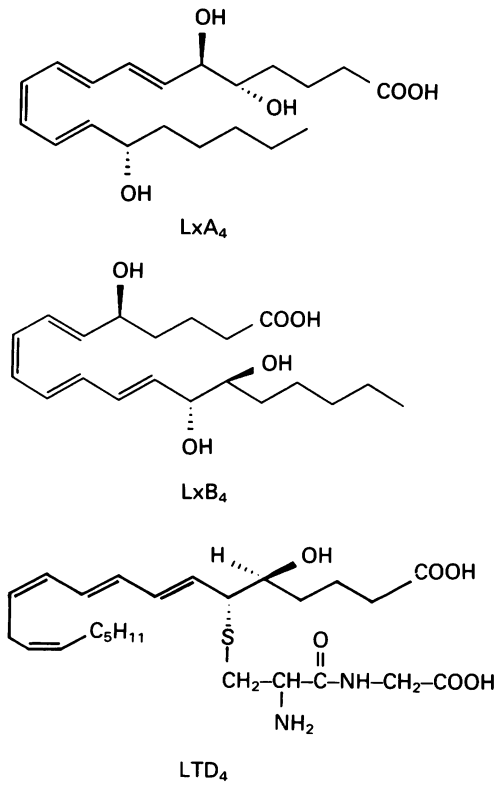

Figure 1 Structures of lipoxin A_4 (LxA_4), lipoxin B_4 (LxB_4) and leukotriene D_4 (LTD_4).

the biological function of these compounds has been due partly to the difficulty in isolating sufficient quantities of lipoxins from biological samples caused by the transformation of metabolites to inactive isomers during the purification process (Serhan *et al.*, 1986a,b; Morris & Wishka, 1986; Spur *et al.*, 1988). We were therefore prompted to prepare LxA_4 and LxB_4 by total chemical synthesis in sufficient quantities for biological evaluation. We have studied the potencies of these compounds in contracting lung tissue and have compared the mechanisms of contractions induced by lipoxins, LTD_4 and LTB_4.

Methods

Tracheal spirals and parenchymal strips were obtained from male guinea-pigs (250–300 g body weight) and they were prepared *in vitro* for recording of isometric contraction after stable baseline tensions had been established as previously described (Lee *et al.*, 1984). The tissues were exposed to logarithmically increasing concentrations of histamine (0.1–100 μM) (Sigma) to construct a cumulative histamine concentration-effect relationship for each tissue preparation. The contraction elicited by 100 μM histamine was assigned to a value of 100 and all subsequent contractile responses to any agonists in that tissue were expressed as a percentage of this reference contraction. After completion of the initial histamine dose-response curve, the tissues were washed with oxygenated Krebs solution maintained at 37°C and aerated with 95% O_2 and 5% CO_2 at 15 min intervals for 45 min. During this time the tension of each tissue returned to that recorded before histamine exposure. The tissues were then used to establish a cumulative dose-response curve for LTD_4, LTB_4, LxA_4 or LxB_4.

Synthetic compounds were prepared by total chemical synthesis (Spur *et al.*, 1988) and their stereochemistries were confirmed by nuclear magnetic resonance. Prior to the bioassays, the concentrations of leukotrienes and lipoxins were checked by ultraviolet absorbance at 280 nm for LTD_4 assuming a molar extinction coefficient (Σ) of 40,000 $cm^{-1}M^{-1}$, at 269 nm for LTB_4 using an Σ of 51,000 $cm^{-1}M^{-1}$; and at 301 nm for the lipoxins using an Σ of 71,000 $cm^{-1}M^{-1}$. The substances were diluted in 9 g $litre^{-1}$ saline solution and added to organ baths to establish cumulative dose-response curves within the range of 0.01 nM–100 nM for LTD_4 and 1 nM–10 μM for LTB_4 and for the lipoxins. In selected experiments, tissues were pre-incubated for 30 min at 37°C with defined concentrations of FPL 55712 (7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylic acid, Fisons), L 649923 (sodium(β5*, γR*)-4-(3-(4-acetyly-3-hydroxy-2-propylphenoxy) propylthic)-γ-hydroxy-β-methylbenzene-butanoate, Merck Frosst), indomethacin (Sigma) or L 651392 (4-bromo-2,7-dimethoxy-3H-phenothiazin-3-one, Merck Frosst) (Young *et al.*, 1986) a 5-lipoxygenase inhibitor (Guindon *et al.*, 1987), prior to the establishment of cumulative dose-response curves to leukotrienes and lipoxins. In separate experiments, parenchymal tissues were pre-incubated for 30 min at 37°C with a combination of 10^{-5} M indomethacin and 10^{-5} M mepyramine in the presence and absence of 10^{-5} M L651392. The tissues were then stimulated to constrict with 0.1 to 10 μM calcium ionophore.

Statistical analysis was performed by Student's *t* test for non paired data.

Results

LxA_4 and LTD_4 elicited a dose-dependent contraction of guinea-pig lung parenchymal strips and the dose-response curves were parallel (Figure 2). The concentration of LxA_4 which produced 50% of the

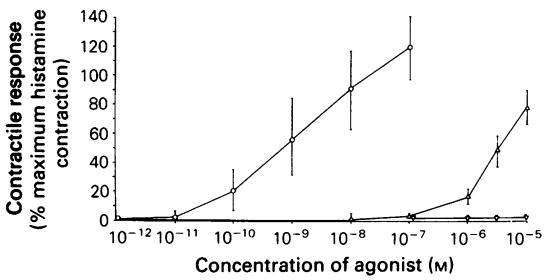

Figure 2 The effect of increasing concentrations of leukotriene D_4 (LTD_4, ○), lipoxin A_4 (LxA_4, △) and lipoxin B_4 (LxB_4, ▽) on contractile responses of guinea-pig parenchymal strips. Each point represents mean of 6, 24 and 8 tissues for the LTD_4, LxA_4 and LxB_4 experiments, respectively; vertical bars show s.d.

maximal histamine response (EC_{50}) was 3×10^{-6} M and the response had not plateaued by 1×10^{-5} M. By interpolation, 5×10^{-10} M LTD_4 would elicit the same response as the EC_{50} of LxA_4, thereby indicating that LTD_4 was approximately 10,000 fold more potent than LxA_4. LxB_4 did not contract parenchymal strips.

The time courses of the contractions produced by LxA_4 and LTD_4 were different from that elicited by histamine. A representative example is shown in Figure 3. Histamine consistently produced a rapid onset of contraction which had plateaued by 2 min. Both LTD_4 and LxA_4 produced contractile reactions which were slow in onset and did not plateau until 20 min.

LTB_4 elicited a dose-dependent contraction of parenchymal strips. The LTB_4 dose-response curve was dissimilar to that of LxA_4 (Figure 4). The concentration of LTB_4 which produced 30% of the maximal histamine response (EC_{30}) and the EC_{50} were 4×10^{-8} M and 8×10^{-7} M, respectively. The EC_{30} and EC_{50} for LxA_4 were 1.5×10^{-6} M and 5×10^{-6} M respectively. In order to assess whether the contractile activity of LxA_4 on parenchymal strips was related to the secondary generation of cyclo-oxygenase products, the contractile activity of LxA_4 was evaluated with and without 10^{-5} M indomethacin pretreatment (Figure 4). There was no significant difference in the contractile response elicited by LxA_4 in the absence and presence of this drug. In contrast, the contractile activity of LTB_4 was completely inhibited by premedication with indomethacin.

The pre-incubation of parenchymal strips with increasing concentrations of FPL 55712 produced a dose-dependent inhibition of the contractile response

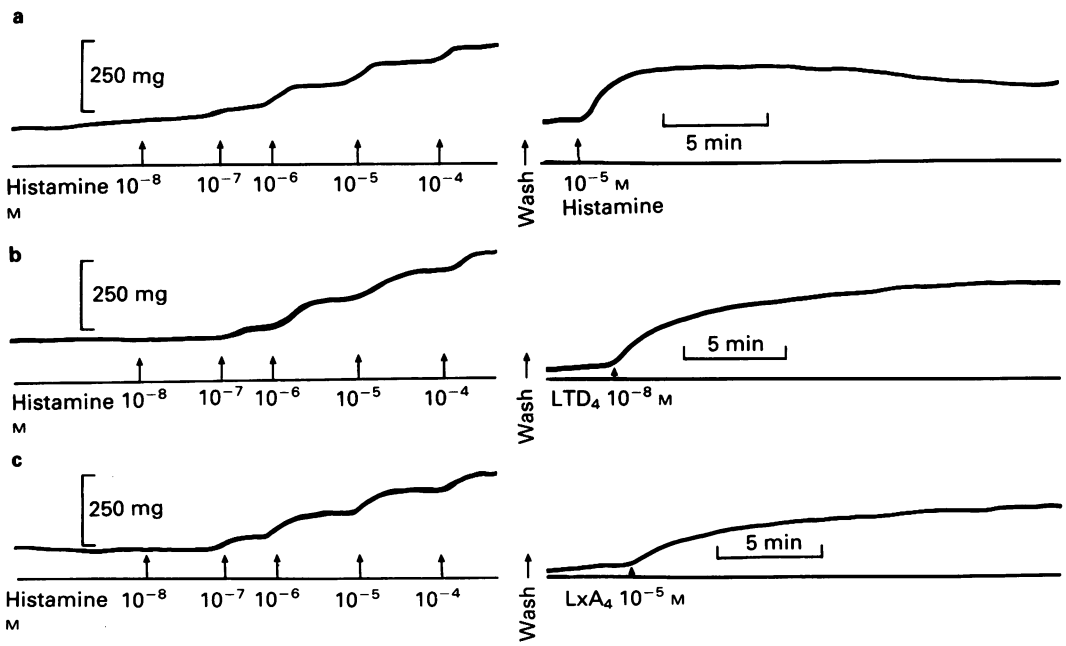

Figure 3 The time course of the contraction elicited by histamine (a), leukotriene D_4 (LTD_4, b) and lipoxin A_4 (LxA_4, c) on guinea-pig parenchymal strips. One representative experiment on three separate tissues is shown. The cumulative histamine concentration-effect relationship for each tissue was initially established (left hand panels). Following washing, the tissues were each exposed to a defined concentration of one of the agonists and the time courses of the contractions were recorded (right hand panels).

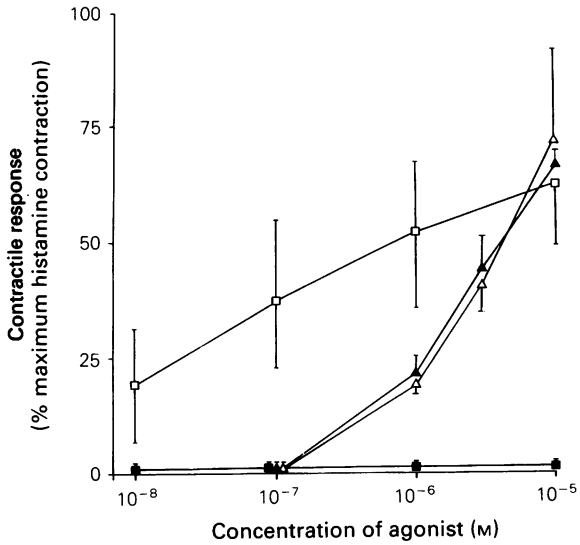

Figure 4 The effect of increasing concentrations of leukotriene B_4 (LTB_4) and lipoxin A_4 (LxA_4) on contractile responses of guinea-pig parenchymal strips, in the absence and presence of 1×10^{-5} M indomethacin. The LTB_4 and LxA_4 dose-response curves constructed without indomethacin pretreatment are indicated by (□) and (△) respectively, and with indomethacin pretreatment are indicated by (■) and (▲), respectively. Each point is the mean of 6 and 5 tissues for the LTB_4 and LxA_4 experiments, respectively; vertical bars show s.d.

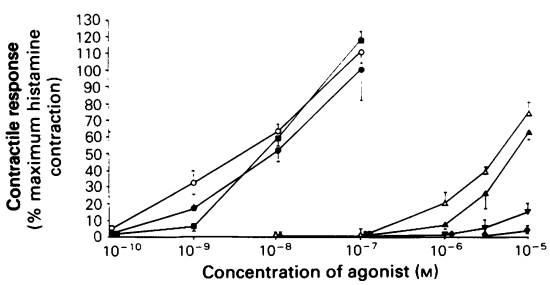

Figure 5 The effect of increasing concentrations of FPL 55712 on the leukotriene D_4 (LTD_4) and lipoxin A_4 (LxA_4) dose-contractile response curves on guinea-pig parenchymal strips. The LTD_4 dose-response curves constructed without and with pretreatment with 1×10^{-6} M and 1×10^{-5} M FPL 55712 are indicated by (○), (●) and (■) respectively. LxA_4 dose-response curves constructed without and with 1×10^{-6} M, 1×10^{-5} M and 3×10^{-5} M FPL 55712 pretreatment are indicated by (△), (▲), (▼) and (◆) respectively. Each point is the mean of 6 and 8 tissues for the LTD_4 and LxA_4 experiments, respectively; vertical bars show s.d.

to all the active concentrations of LxA_4 studied (Figure 5). There was a parallel displacement of the LxA_4 dose-response curve to the right by 10^{-6} M FPL 55712. 1×10^{-6} M and 1×10^{-5} M, FPL 55712 produced a significant inhibition of the contractile activity of 10^{-9} M LTD_4 only ($P = 0.002$ and $P = 0.001$, respectively) and the inhibition was not seen at higher concentrations of LTD_4 (Figure 5).

Preincubation of parenchymal strips with 3×10^{-5} M L 649923 produced an inhibition of the contractions elicited by LxA_4 at all the concentrations studied (Figure 6). Pretreatment of lung tissue with the same dose of L 649923 produced an inhibition of the contractions induced by 5×10^{-9} M to 3×10^{-8} M LTD_4 (Figure 6).

In separate experiments, histamine dose-response curves were constructed in the presence or absence of either 3×10^{-5} M FPL 55712 or 3×10^{-5} M L 649923. There was no difference in the histamine dose-response curves between any of these experiments, indicating that this concentration of FPL 55712 and L 649923 did not have a non-specific spasmolytic action. The EC_{50} for histamine of control, FPL 55712- and L 649923-treated tissues were 1.9×10^{-6} M (mean, $n = 4$), 1.8×10^{-6} M (mean, $n = 6$) and 2.1×10^{-6} M (mean, $n = 3$), respectively.

The incubation of parenchymal strips with 10^{-6} M and 10^{-5} M L 651392, a 5-lipoxygenase inhibitor, produced no inhibitory effect on the activity of LxA_4 (Figure 7a). In separate experiments as controls, parenchymal strips were pretreated with 1×10^{-5} M indomethacin and 1×10^{-5} M mepyramine in the presence or absence of 1×10^{-5} M L 651392, a 5-lipoxygenase inhibitor. The tissues were then stimulated to contract in a dose-dependent manner with 0.1–10.0 μM calcium ionophore. In those tissues that were pre-incubated with indomethacin and mepyramine, in order to uncover the 5-lipoxygenase, and then were subsequently contracted by ionophore, the EC_{50} was 4.4×10^{-6} M. The EC_{50} of tissues treated with L 651392, indomethacin and mepyramine and then contracted by ionophore was 1×10^{-5} M (Figure 7b).

LxA_4 and LxB_4 did not contract tracheal spirals. LTD_4 produced a dose-dependent contraction of tracheal smooth muscle and the EC_{50} was 3×10^{-8} M.

Discussion

A novel series of arachidonic acid derived metabolites produced through the interaction of the 5- and 15-lipoxygenase pathways have been evaluated on guinea-pig lung tissue. The biologically active compounds were prepared by total organic synthesis and

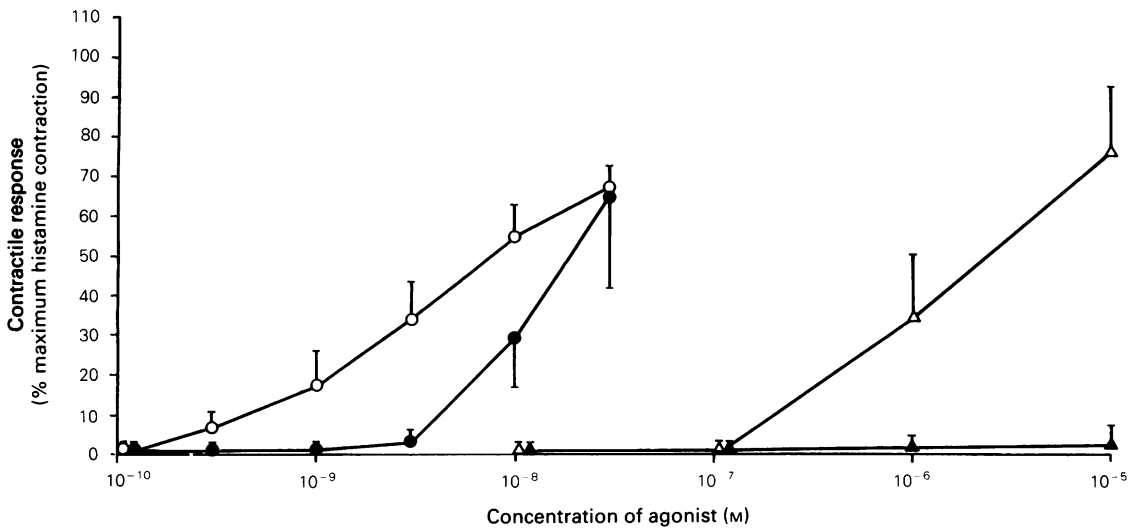

Figure 6 The effect of 3×10^{-5} M L 649923 on the leukotriene D_4 (LTD_4) and lipoxin A_4 (LxA_4) dose-contractile response curves on guinea-pig parenchymal strips. The LTD_4 dose-response curves constructed in the absence and presence of 3×10^{-5} M L 649923 are indicated by (○) and (●), respectively. LxA_4 dose-response curves constructed in the absence and presence of 3×10^{-5} M L 649923 are indicated by (△) and (▲), respectively. Each point is the mean of 8 and 4 tissues for the LTD_4 and LxA_4 experiments respectively; vertical bars show s.d.

their authenticities were confirmed unequivocally by nuclear magnetic resonance and comparisons with biologically derived materials (Nicolaou *et al.*, 1985; Nicolaou & Webber, 1986). The results indicate that LxA_4, but not LxB_4, possessed contractile activity for guinea-pig lung parenchymal strips but not for tracheal smooth muscle over the concentration-range studied. These findings suggest that the presence of the hydroxyl groups at C-5 and C-6 positions of LxA_4 is critical for the expression of contractile activity. LxA_4 and LTD_4 demonstrated dose-contractile response curves which were parallel but LxA_4 was substantially less potent than LTD_4 in contracting parenchymal strips. The time course of the response to LxA_4 was virtually identical to that of LTD_4. LTB_4 was more potent than LxA_4 and the dose-response curves elicited by LTB_4 and LxA_4 were dissimilar. The contractile response to LxA_4 was not inhibited by 10^{-5} M indomethacin, a concentration that inhibited the cyclo-oxygenase product-dependent contraction produced by LTB_4 (Sirois *et al.*, 1981). These results indicate that LxA_4 activity was not mediated through the secondary generation of cyclo-oxygenase metabolites.

Since the time courses of the contractile response induced by LxA_4 and LTD_4 were very similar, we were prompted to evaluate the effects of two LTD_4 antagonists, FPL 55712 and L 649923, on the activity of LxA_4 and LTD_4. FPL 55712 and L 649923 inhibited LxA_4 action throughout the whole range of doses studied suggesting that LxA_4 may act through the LTD_4 receptor. The finding that FPL 55712 and L 649923 had no effect on histamine-induced contraction indicates that the inhibitory effect of these antagonists on LxA_4 action observed in this study was not due to a non-specific spasmolytic effect of these inhibitors.

Previous work has suggested that there may be different LTD_4 receptors in smooth muscle (Drazen *et al.*, 1980; Fleisch *et al.*, 1982b; Krell *et al.*, 1983). Drazen *et al.* demonstrated that FPL 55712 inhibited the contractile response in guinea-pig lung parenchymal strips at low concentrations of LTD_4, which produced less than 30 to 40% of the maximal histamine contractile activity, but that it was without effect on the spasmogenic response to higher concentrations of LTD_4 (Drazen *et al.*, 1980). Our results confirm these observations. The differential effect of FPL 55712 on the contractions induced by low and high concentrations of LTD_4 in lung parenchymal strips has been interpreted as suggesting the presence of two classes of LTD_4 receptors in this tissue; a high affinity receptor that is inhibited by FPL 55712 and a low affinity receptor that is not affected by this antagonist (Drazen *et al.*, 1980). On the basis of our results with FPL 55712, the data would be consistent

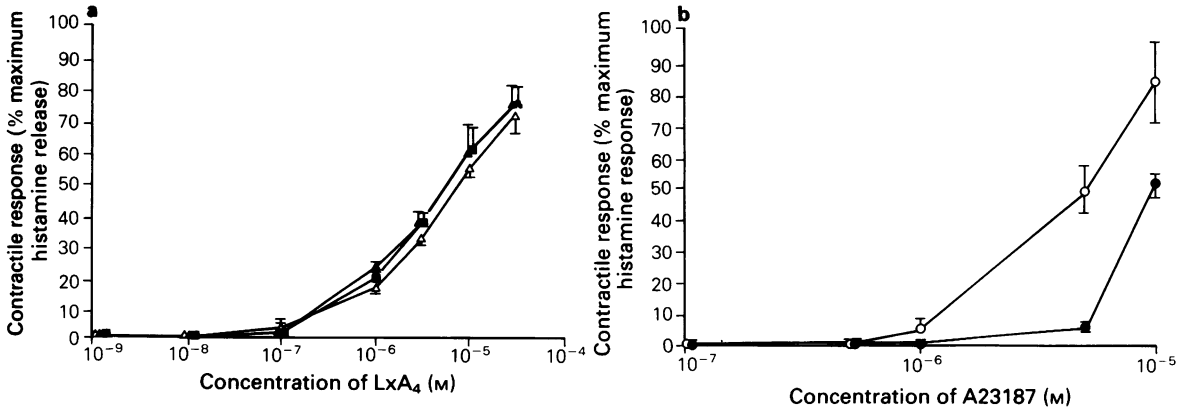

Figure 7 The effect of increasing concentrations of L 651392 on the lipoxin A_4 (LxA_4, a) and calcium ionophore (A23187) (b) dose-response curves on guinea-pig parenchymal strips which had been pretreated with 1×10^{-5} M indomethacin and 1×10^{-5} M mepyramine. The LxA_4 dose-response curves constructed without and with pretreatment with 1×10^{-6} M and 1×10^{-5} M L 651392 are indicated by (△), (▲) and (■) respectively. The calcium ionophore dose-response curves constructed in the absence and presence of 1×10^{-5} M L 561392 are indicated by (○) and (●) respectively. Each point is the mean of 6 and 3 tissues for the LxA_4 and calcium ionophore experiments, respectively; s.d. shown by vertical lines.

with the view that LxA_4 mediates its contractile activity through a similar mechanism to that utilised by low concentrations of LTD_4.

In order to evaluate whether LxA_4 might have produced a contractile effect through the secondary release of SRS-A leukotrienes, LxA_4 dose-response curves were constructed in the absence and presence of a selective 5-lipoxygenase inhibitor, L 651392 (Guindon *et al.*, 1987). This had no effect on LxA_4 action, even though it partially inhibited the calcium ionophore-induced contraction of parenchymal tissue, which had been pretreated with an antihistamine and a cyclo-oxygenase inhibitor to uncover the lipoxygenase contribution (Fleisch *et al.*, 1982a). These results indicate that LxA_4 did not produce contractions through the secondary release of the sufidopeptide leukotrienes.

In conclusion, LxA_4 but not LxB_4 has intrinsic spasmogenic activity which is selective for guinea-pig lung parenchymal strips over the concentration-range studied. The activity of LxA_4 is slow in onset and long lasting. It is substantially less potent than LTD_4 and may exert its effects on airway smooth muscle via the putative high affinity LTD_4 receptor.

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