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STIMULATION OF ARACHIDONIC ACID METABOLISM AND GENERATION OF THROMBOXANE A_2 BY LEUKOTRIENES B_4, C_4 AND D_4 IN GUINEA-PIG LUNG *in vitro*

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1 Leukotriene C_4 (LTC_4), LTD_4, slow-reacting substance of anaphylaxis (SRS-A) (from guinea-pig lung), bradykinin (Bk) and arachidonic acid (AA) release thromboxane A_2 (TxA_2) and prostaglandin-like materials from guinea-pig isolated perfused lungs.

2 Release of TxA_2 induced by LTC_4 and LTD_4 is inhibited by a thromboxane synthetase inhibitor, imidazole (2.9 mM).

3 Mepacrine (200 μM), a phospholipase inhibitor, inhibits release of TxA_2 and prostaglandin-like materials caused by SRS-A and Bk but not that due to exogenous AA

4 Leukotrienes B_4, C_4 and D_4 are approximately equipotent in inducing dose-related contractions of guinea-pig parenchymal strips (GPPs).

5 Leukotriene-induced contractions of GPPs are greatly inhibited by imidazole (2.9 mM), carboxyheptylimidazole (24 μM) and mepacrine (400 μM).

6 FPL 55712 (1.9 μM), the SRS-A antagonist, blocks contractions of GPPs induced by LTC_4 and LTD_4 but not those due to LTB_4 or Bk.

7 Tachyphylaxis to LTB_4 occurs in GPPs but not to LTC_4 or LTD_4.

8 These results suggest that in guinea-pig lung *in vitro*, LTB_4, LTC_4 and LTD_4 activate a phospholipase with subsequent generation of cyclo-oxygenase products of which TxA_2 plays an important role.

Introduction

Leukotriene D_4 (LTD_4) has been shown to be the major biological activity of slow-reacting substance of anaphylaxis (SRS-A) when measured on guinea-pig ileum (Morris, Taylor, Piper & Tippins, 1980; Morris, Taylor, Rokach, Girard, Piper, Tippins & Samhoun, 1980). LTD_4 is probably formed from LTC_4 by γ-glutamyltranspeptidase (Örning & Hammarström, 1980) and these leukotrienes have biological actions that are very similar to those of slow-reacting substance of anaphylaxis. However, there are differences in potency and duration of action between LTC_4 and LTD_4 in some systems (Piper, Samhoun, Tippins, Williams, Palmer & Peck, 1981; Letts & Piper, 1981). In addition to LTD_4, perfusates from guinea-pig lung collected during anaphylaxis contained appreciable amounts of another leukotriene, LTB_4 (Morris, Taylor, Piper & Tippins, 1979) which, unlike LTC_4 and LTD_4, lacks the peptide side chain at C-6 (Radmark, Malmsten, Samuelsson, Clark, Goto, Marfat & Corey, 1980).

Slow-reacting substance of anaphylaxis and bradykinin (Bk) are potent bronchoconstrictor agents in guinea pig *in vivo*. Furthermore, both SRS-A- and Bk-induced bronchoconstriction in this species is inhibited by aspirin-like drugs (Collier, Holgate, Schachter & Shorley, 1960; Berry & Collier, 1964). Since non-steroid anti-inflammatory drugs inhibit prostaglandin synthesis (Vane, 1971), this suggests that cyclo-oxygenase products of arachidonic acid (AA) metabolism have a role in the airways constriction due to SRS-A and bradykinin. Further, both SRS-A and Bk release rabbit aorta-contracting substance (RCS) from guinea-pig isolated perfused lungs (Piper & Vane, 1969). The release of RCS is inhibited by aspirin (Palmer, Piper & Vane, 1973) and glucocorticoids (Engineer, Morris, Piper & Sirois, 1978). The major component of RCS has been shown to be the unstable AA metabolite, thromboxane A_2 (TxA_2) ($T_{\frac{1}{2}}$ 30–40s at 37°C) which causes constriction of arteries, airway smooth muscle and platelet aggregation (Svensson, Hamberg & Samuelsson, 1975; Samuelsson, 1976; Svensson, Strandberg, Tuvemo & Hamberg, 1977). In this paper, the arterial smooth muscle-contracting material ($T_{\frac{1}{2}} < 60$s, 37°C) released from guinea-pig lung by various agonists will be referred to as thromboxane

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A_2. Like SRS-A, LTC_4 and LTD_4 release cyclo-oxygenase products from guinea-pig isolated perfused lung and contract guinea-pig parenchyma: both actions are blocked by indomethacin (Piper & Samhoun, 1981). Although LTB_4 has little effect on gastrointestinal or vascular smooth muscle, it contracts guinea-pig parenchyma and these contractions are also inhibited by indomethacin (Sirois, Borgeat, Jeanson, Roy & Girard, 1980).

The purpose of the investigations described in this paper was to assess the contribution of TxA_2 to the actions of the leukotrienes in guinea-pig perfused lungs and parenchyma *in vitro*. Since the Bk-induced release of cyclo-oxygenase products from guinea-pig lung is also inhibited by mepacrine (Vargaftig & Dao Hai, 1972), we have investigated the effect of mepacrine on the mechanism of release of TxA_2 by leukotrienes.

Methods

Male guinea-pigs (Dunkin-Hartley) weighing 500–700 g were used, and their lungs and ilea were excised after cervical dislocation. Other assay tissues were obtained from male rabbits (New Zealand White strain, 3–3.5 kg) and male rats (Wistar strain, 250–300 g).

Isolated perfused lungs and assay tissues

Lungs were inflated via the trachea and perfused free from blood with oxygenated Tyrode solution (5 ml/min) at 37°C via the pulmonary artery. Lung effluent superfused a series of assay tissues sensitive to cyclo-oxygenase products and to LTC_4 and LTD_4 (SRS-A). The tissues were strips of: rabbit aorta (RbA), rabbit mesenteric artery (RbMA), rat stomach (RSS) and sometimes guinea-pig ileum smooth muscle (GPISM). They were superfused continuously with mepyramine (0.1 μg/ml), hyoscine (0.1 μg/ml), methysergide (0.2 μg/ml), phenoxybenzamine (0.1 μg/ml) and propranolol (2 μg/ml) (Piper & Vane, 1969).

Lung parenchymal strips

Lungs were perfused free from blood as described above, and sections of parenchyma were prepared by a modification of the method of Lulich, Mitchell & Sparrow (1976). Strips of lung (30 × 3 × 3 mm) containing no pleura were selected from the large lobes distal to the large airways, and were suspended in a cascade system under a tension of 1 g. They were superfused at 37°C with oxygenated Tyrode solution (5 ml/min) containing the same mixture of antagonists as above.

Preparation of drugs

All drugs used in this study were prepared in Tyrode solution except FPL 55712 (distilled water). Stock solutions of AA, LTC_4, LTD_4, and U-44069 were prepared in ethanol; those of LTB_4 in methanol. Alcohol was evaporated and the required dilutions made in Tyrode solution. Imidazole solutions were adjusted to pH 7.4 with 0.1 N HCl.

Administration of drugs:

Isolated perfused lungs: Agonists, i.e. AA, Bk, SRS-A, LTC_4 and LTD_4 were administered as bolus injections (10–100 μl) either into the effluent superfusing the assay tissues directly or intra-arterially through the lungs.

Imidazole: Imidazole was superfused directly over the assay tissues while different doses of LTC_4 and LTD_4 were administered intra-arterially. Imidazole was then infused into the lungs for 20 min before and then continuously during re-testing the different doses of LTC_4 and LTD_4. U-44069 ((15S)-hydroxy-9α, 11α-(epoxymethano)prosta-5Z, 13E-dienoic acid), a stable prostaglandin endoperoxide analogue which is a bronchoconstrictor (Wasserman, 1976) and has TxA_2-like actions on smooth muscle preparations, was used as a standard agonist to test the sensitivity of the assay tissues.

Mepacrine: Mepacrine did not affect RbA, RbMa or RSS but altered the sensitivity of GPISM. Dose-response curves to SRS-A, AA and Bk given intra-arterially were therefore obtained in the absence of mepacrine. Mepacrine was then infused intra-arterially for 10–15 min and the lung effluent directed away from the assay tissues which in the meantime were superfused with mepacrine-free Tyrode. At the end of the infusion of mepacrine, the lung effluent was again superfused over the assay tissues and the different doses of agonists were then re-tested.

Parenchymal strips: LTB_4, LTC_4, LTD_4 and Bk were administered as bolus injections (10–100 μl) into the fluid superfusing the guinea-pig parenchymal strips (GPPs). In all experiments, U-44069 was used as a standard agonist to test the sensitivity of the parenchymal strips. To prevent tachyphylaxis from occurring doses of LTB_4 were always alternated with doses of other agonists. Actions of agonists were studied before and after administration of antagonists and inhibitors.

FPL55712: FPL55712 was superfused over the parenchymal strips for 10 min before and then continuously during re-testing of LTB_4, LTC_4, LTD_4 and Bk.

Imidazole, carboxyheptylimidazole and mepacrine: Parenchymal strips were superfused with either im-

idazole, carboxyheptylimidazole or mepacrine for 20 min before and continuously during re-testing of LTB_4, LTC_4 or LTD_4. In one series of experiments comparing the effect of imidazole on the actions of LTB_4 and LTD_4, strips of GPISM were included in the superfusion cascade and were suspended below the parenchymal strips.

Drugs

The following were used: arachidonic acid (Grade I, Sigma); bradykinin triacetate (Sigma); hyoscine hydrobromide (BDH); imidazole (Grade III, Sigma); mepacrine B.P., mepyramine maleate (May & Baker); methysergide maleate (Sandoz); phenoxybenzamine hydrochloride and propranolol hydrochloride (I.C.I.). SRS-A from guinea-pig lung was prepared by the method of Engineer *et al.* (1978). Naturally-occurring LTB_4 obtained from rat polymorphonuclear leucocytes (PMNs) was a gift from Dr A. W. Ford-Hutchinson. The following compounds were gifts from companies shown in parentheses: carboxyheptylimidazole (Ciba-Geigy); sodium 7-[3(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate (FPL 55712, Fisons); U-44069 (The Upjohn Company); synthetic leukotrienes (LTB_4, LTC_4 and LTD_4) with stereochemistry of naturally-occurring compounds (Merck Frosst Laboratories).

Statistical methods

The mean and standard error of the mean were calculated for experiments describing results obtained using parenchymal strips. Each parenchymal strip was taken from a separate lung and therefore the *n* values indicate numbers of parenchymal strips or perfused lungs used in the different series of experiments.

Results

Release of cyclo-oxygenase products from guinea-pig isolated perfused lungs and its inhibition

When SRS-A, LTC_4, LTD_4, Bk or AA were injected into guinea-pig isolated perfused lungs and the effluent superfused directly over RbA, RbMA and RSS, these tissues contracted. However, when the effluent was delayed by 2 min the contractile activity on the arterial tissues had almost disappeared but the contraction of RSS was only partially reduced. Release of TxA_2 was detected by contractions of RbA and RbMA and that of prostaglandin-like material by contractions of RSS. In all experiments this release of cyclo-oxygenase products induced by SRS-A and the LTs was reproducible and did not decline over the duration of individual experiments.

Effect of imidazole: LTC_4 and LTD_4 were found to be equipotent in releasing TxA_2 and prostaglandin-like materials from isolated perfused lungs. Imidazole (2.9 mM) infused intra-arterially reduced the dose-related contractions of RbA and RbMA induced by LTC_4 and LTD_4 (both administered at 1–5 pmol) by 70–100% and contractions of RSS by approximately 50%. When higher doses of LTC_4 or LTD_4 (7–10 pmol) were administered the effect of imidazole was overcome. Contractions of RbA and RbMA due to 10 pmol of LTC_4 or LTD_4 were approximately equivalent to those obtained by 3 pmol administered prior to treatment of the lungs with imidazole. However, under the same conditions, contraction of RSS was larger ($n = 4$ in all cases). At the doses used, LTC_4 and LTD_4 administered directly over the tissues did not contract the arterial tissues but produced small contractions of the RSS. Contractions of the RbA, RbMA and RSS due to U-44069 given directly to the assay tissues were stable and reproducible and were not affected by imidazole. Typical results obtained on RbA and RSS, using LTD_4 appear in Figure 1.

Effect of mepacrine:

SRS-A: Mepacrine (200 μM) infused intra-arterially into isolated perfused lungs inhibited the dose-related contractions of RbA and RbMA and greatly reduced those of RSS induced by i.a. administrations of SRS-A (0.2–1.0 u, the equivalent of 2–10 pmol of LTD_4). The effect of mepacrine was reversible because 35 min after termination of the mepacrine infusion, RbA, RbMA and RSS contracted, indicating that the release of TxA_2 and prostaglandin-like materials from the lungs had completely returned ($n = 4$). The direct effect of SRS-A was measured on GPISM which was included in the superfusion cascade system. Contractions of GPISM to SRS-A given intra-arterially throughout the experiment were much smaller than those due to doses administered directly, showing loss of biological activity of SRS-A during passage through the pulmonary vascular bed. SRS-A administered directly over the tissues did not contract the arterial tissues but produced small contractions of the RSS. Typical results obtained on RbA and GPISM are shown in Figure 2. At the same doses of SRS-A, similar results were obtained with mepacrine at 400 μM ($n = 3$). However, at this higher concentration, mepacrine increased the spontaneous activity of GPISM. No inhibition of SRS-A-induced release of cyclo-oxygenase products was seen with mepacrine at 100 μM ($n = 3$).

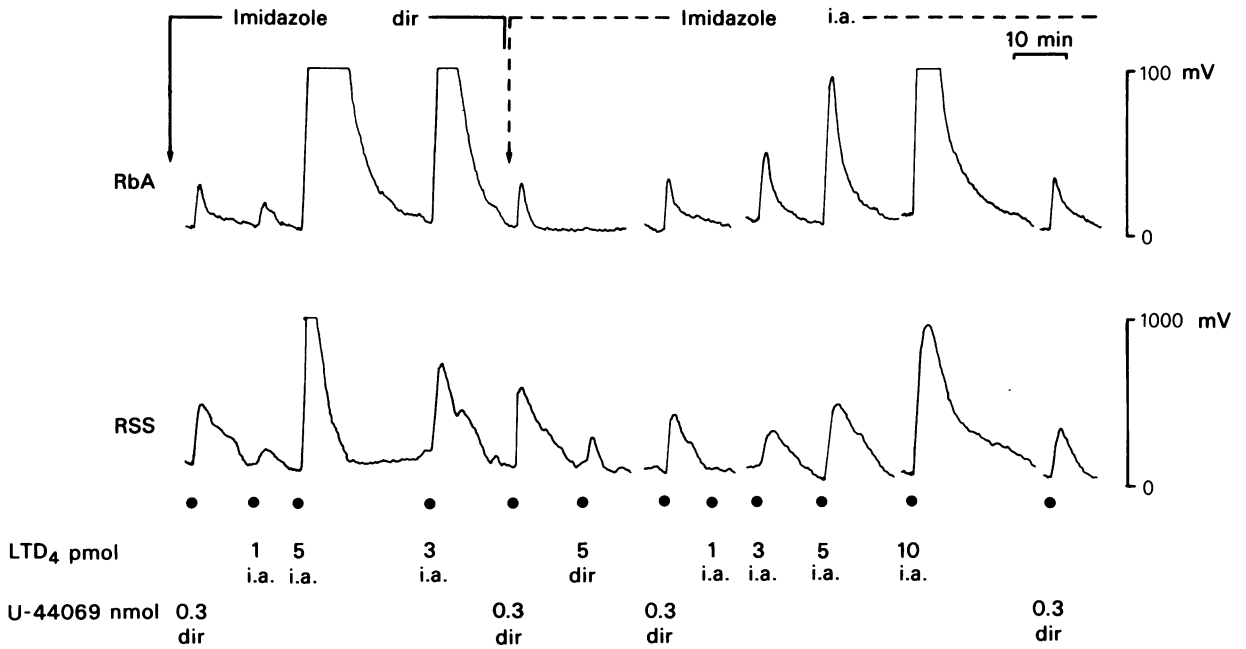

Figure 1 The effects of imidazole (2.9 mM) on the release of thromboxane A_2 (TxA_2) and prostaglandin-like materials from guinea-pig isolated perfused lungs induced by leukotriene D_4 (LTD_4). Upon administration of intra-arterial (i.a.) doses of LTD_4 (1, 5 and 3 pmol) dose-related contractions of RbA (showing release of TxA_2) and RSS (showing mainly release of prostaglandin-like materials) were obtained. After i.a. infusion of imidazole, contractions of RbA and RSS to the same doses of LTD_4 were reduced. LTD_4 (5 pmol) given directly (dir) over the tissues did not contract the RbA and produced only a small contraction of the RSS.

Contractions to U-44069 (0.3 nmol) given dir were stable and were not inhibited by imidazole. Horizontal scale: 10 min. Vertical scale: mV.

Bradykinin: Mepacrine (200 μM) also reversibly inhibited the release of TxA_2 and prostaglandin-like substances induced by i.a. administration of Bk (5–10 nmol). Bradykinin had no direct effect on the RbA or RbMA but produced dose-related contractions of the RSS which were smaller than the contractions of this tissue due to the same doses of Bk administered into the lungs ($n = 3$).

Arachidonic acid: Mepacrine (200 μM and 400 μM) did not inhibit the subsequent release of TxA_2 and prostaglandin-like materials induced by i.a. administrations of AA (15–80 nmol). Arachidonic acid administered directly over the tissues did not contract the RbA, RbMA or RSS but throughout the experiment produced very small contractions of GPISM ($n = 4$). Typical results obtained on RbA and GPISM are shown in Figure 2.

Leukotriene-induced contractions of guinea-pig parenchymal strips

Leukotrienes B_4, C_4 and D_4, Bk and U-44069 induced dose-related contractions of guinea-pig parenchymal strips which were stable and reproducible during individual experiments as well as between experiments. When compared on the same GPPs in various studies, LTB_4, LTC_4 and LTD_4 were found to be approximately equipotent. Tachyphylaxis was observed after successive administrations of LTB_4 but not after LTC_4 or LTD_4. Doses of LTB_4 were therefore administered alternately with doses of other agonists. Preliminary results using radioimmunoassay showed the presence of TxB_2 in the superfusion fluid collected during contraction of parenchymal strips due to LTB_4 and LTD_4.

Effect of FPL 55712 (1.9 μM): FPL 55712 antagonized the contractions of GPPs induced by LTC_4 and LTD_4 (both administered at 1–30 pmol) but not those due to LTB_4 (1–30 pmol), Bk (50–500 pmol) or U-44069 (0.3–3.0 nmol) ($n = 10$ in all cases). Typical results using LTB_4 and LTC_4 are shown in Figure 3.

Effect of imidazole (2.9 mM): Imidazole reduced by 75–100% the contractions of GPPs due to LTB_4

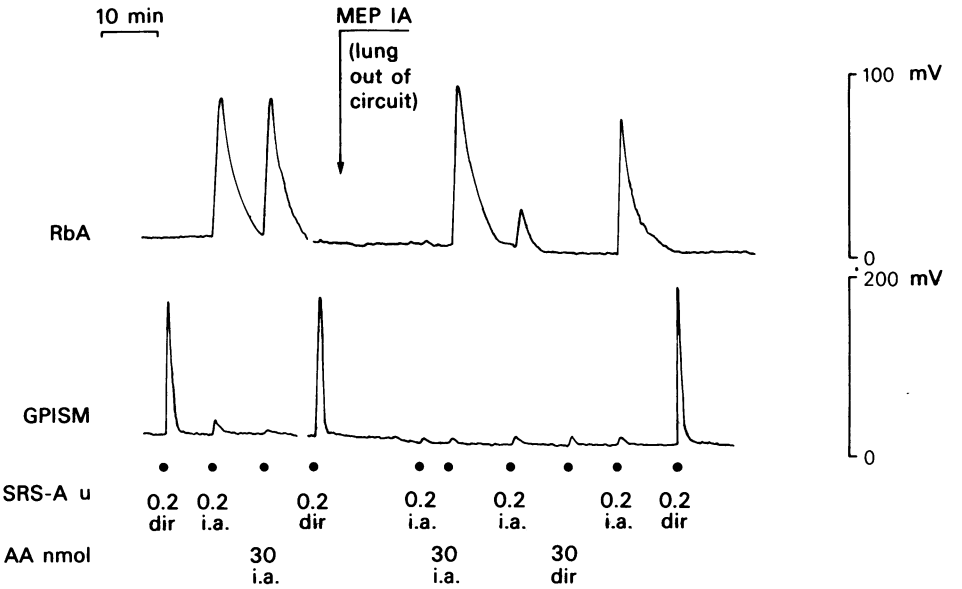

Figure 2 The effect of mepacrine (200 μM) on the slow-reacting substance of anaphylaxis (SRS-A)-induced release of thromboxane A_2 (TxA_2). After mepacrine, contractions of RbA following i.a. dose of SRS-A (0.2 u) were inhibited while contractions due to arachidonic acid (AA, 30 nmol) were unchanged. The SRS-A-induced release of TxA_2 returned to its original level after 35 min. SRS-A given Dir induced reproducible contractions of GPSIM but did not contract RbA. Smaller contractions of GPISM were obtained following i.a. doses of SRS-A, showing loss of activity in the pulmonary circulation. AA (30 nmol) caused a small contraction of GPISM but had no effect on RbA. Horizontal scale: 10 min. Vertical scale: mV.

(derived from PMNs) and LTD_4 (both administered at 1–30 pmol) but not those due to U-44069 (0.3–3.0 nmol). Contractions of GPPs due to 10 pmol of LTB_4 and LTD_4 were reduced by 78.5 ± 8.2% and 72.7 ± 4.6% respectively. LTD_4 produced dose-related contractions of GPISM which were reproducible and resistant to imidazole. Unlike LTD_4, LTB_4 and U-44069 did not contract the GPISM ($n = 4$ in all cases). Typical results are shown in Figure 4.

In another series of experiments comparing the actions of LTC_4 and LTD_4 (both administered at

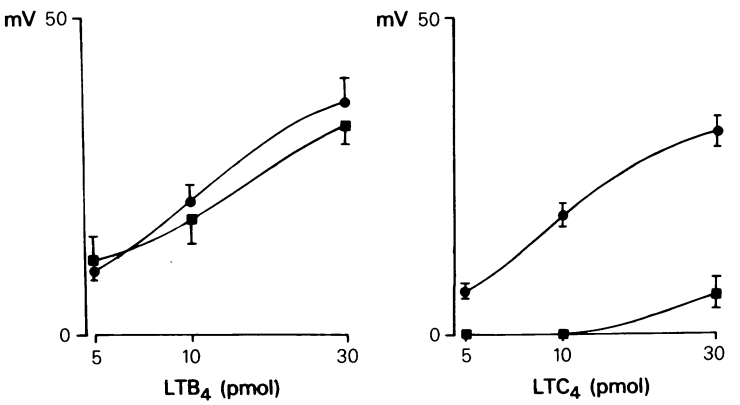

Figure 3 Effect of FPL 55712 (1.9 μM) on contractions induced by leukotriene B_4 (LTB_4) and LTC_4 (both at 5–30 pmol) on the same guinea-pig parenchymal strips (GPPs). FPL 55712 (■) reduced the LTC_4-induced contractions of GPPs (●) (right-hand panel) but did not reduce those due to LTB_4 (left-hand panel). Bars represent s.e.mean from 8 experiments. Ordinates: 50 mV. Abscissae: doses of LTB_4, LTC_4 (pmol).

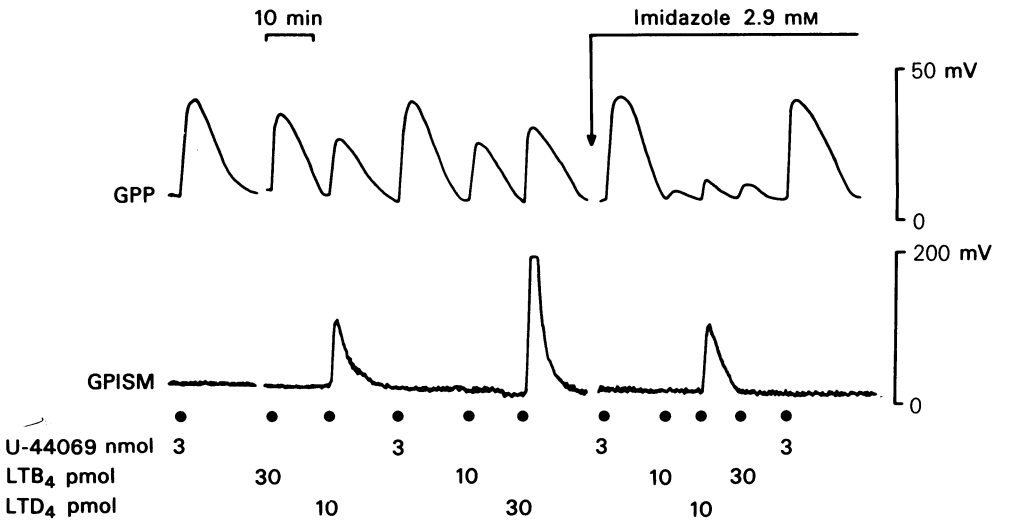

Figure 4 Effect of imidazole (2.9 mM) on responses of strips of guinea-pig parenchyma (GPP) and guinea-pig ileum smooth muscle (GPISM) induced by leukotriene B_4 (LTB_4), LTD_4 (both at 10 and 30 pmol) and U-44069 (3 nmol). Imidazole (at arrow) superfused over the tissues considerably reduced the contractions of GPP due to the LTs but not those to U-44069. Contractions of GPISM due to LTD_4 were not inhibited by imidazole. Vertical scale: mV. Horizontal scale: 10 min.

1–10 pmol), imidazole inhibited the leukotriene-induced contractions of GPPs by 70–95% ($n = 5$ in all cases). Contractions elicited by 5 pmol of LTC_4 and LTD_4 were reduced by $81.7 \pm 3.1\%$ and $84.2 \pm 2.1\%$ respectively.

Effect of carboxyheptylimidazole (24 μM): Carboxyheptylimidazole produced a 63–82% inhibition of contractions induced by LTC_4 and LTD_4 (both at 1–10 pmol) on the same parenchymal strips. Contractions of GPPs due to 10 pmol of LTC_4 and LTD_4

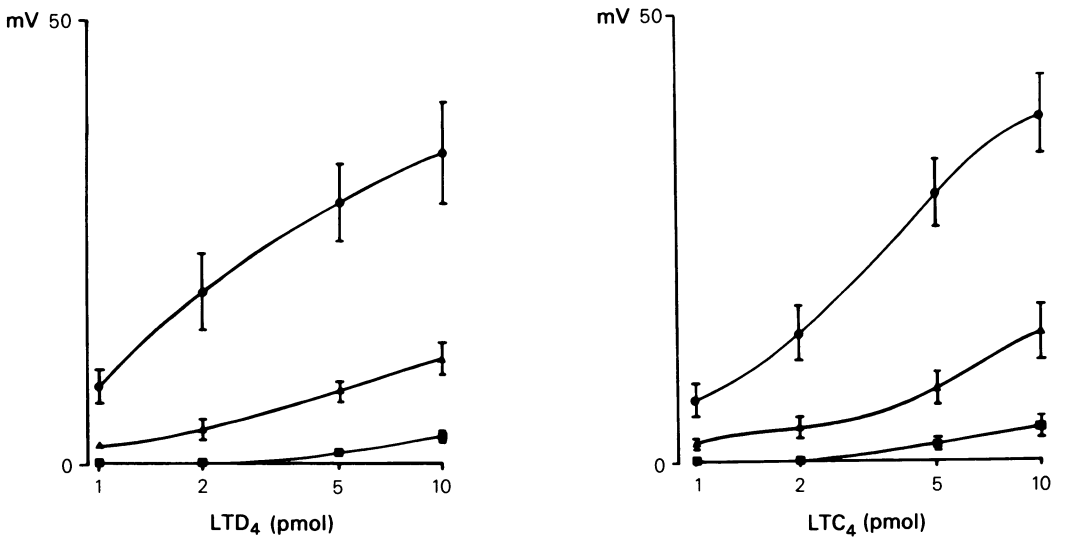

Figure 5 Inhibition of contractions of guinea-pig parenchymal strips due to 1–10 pmol of leukotriene D_4 (LTD_4) and LTC_4 (left and right-hand panels respectively) by carboxyheptylimidazole (CHI, 24 μM) alone (▲) and CHI plus FPL 55712 (1.9 μM). Bars represent s.e.mean from 8 experiments. Ordinates: 50 mV. Abscissae: doses of LTD_4, LTC_4 (pmol).

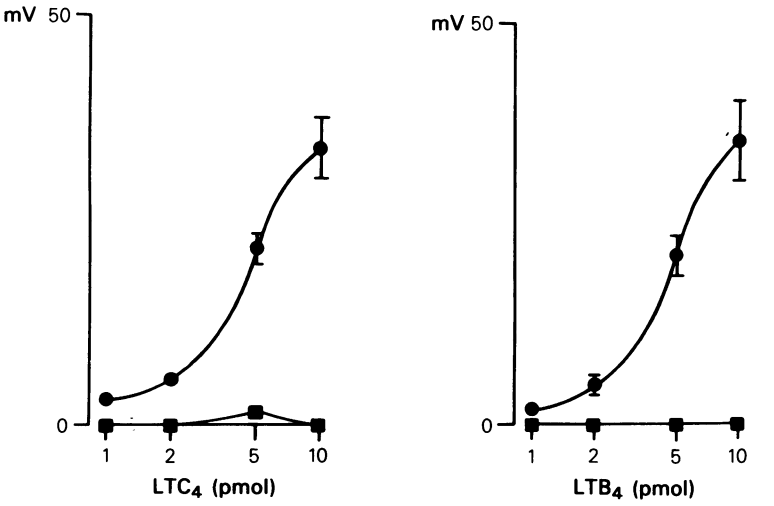

Figure 6 Effect of mepacrine (400 μM) on contractions induced by leukotriene B_4 (LTB_4) and LTC_4 (both at 1–10 pmol) on the same guinea-pig parenchymal strips (GPPs). Mepacrine (Mep) (■) inhibited the contractions of GPPs caused by LTB_4 and LTC_4 (●) (left- and right-hand panels respectively). Bars represent s.e.mean from 6 experiments. Ordinates: 50 mV. Abscissae: doses of LTB_4, LTC_4 (pmol).

were reduced by 64.7 ± 4.9% and 64.4 ± 2.2% respectively. FPL 55712 (1.9 μM) superfused over the tissues in the presence of carboxyheptylimidazole further reduced the residual contractions of GPPs to LTC_4 and LTD_4 ($n = 8$ in all cases) (Figure 5).

In another series of experiments, carboxyheptylimidazole inhibited by 63–89% the contractions of GPPs induced by LTB_4 (5–50 pmol). Contractions due to 10 pmol were reduced by 87.5 ± 3%. However, FPL 55712 administered in the presence of carboxyheptylimidazole did not reduce further the residual contractions of the GPPs. Contractions of GPPs due to U-44069 (0.3–3.0 nmol) were unaffected by either carboxyheptylimidazole or FPL 55712.

Effect of mepacrine (400 μM): Mepacrine inhibited the contractions of GPPs induced by LTB_4, LTC_4 and LTD_4 (all administered at 1–10 pmol) but not those due to U-44069 (0.3–3.0 nmol). Typical results using LTB_4 and LTC_4 are shown in Figure 6.

This inhibition was slowly reversible and 90 min after termination of mepacrine infusion, a 50% recovery of contractions of GPPs to the leukotrienes was observed ($n = 6–12$).

Discussion

The experiments described in this paper show that, like SRS-A, LTB_4, LTC_4 and LTD_4 stimulate arachidonic acid metabolism in isolated guinea-pig lung and parenchymal strips. This action of SRS-A and the leukotrienes is reversibly inhibited by mepacrine, a phospholipase inhibitor, which has no effect on the generation of TxA_2 and prostaglandin-like materials induced by exogenous AA from isolated perfused lungs. This suggests that, by supplying the lung with the substrate (i.e. AA), TxA_2 and prostaglandin synthesis can occur independently of a phospholipase. However, in the case of SRS-A and the leukotrienes stimulation of phospholipase is an essential step for the subsequent release of endogenous AA and generation of TxA_2.

Inhibition by mepacrine of the Bk-induced release of cyclo-oxygenase products from isolated perfused lungs suggests that Bk also exerts its action via activation of phospholipase (as previously described by Vargaftig & Dao Hai (1972)).

Further evidence for the stimulation of phospholipase by SRS-A can be inferred from studies describing the inhibition of the effect of SRS-A in guinea-pig isolated perfused lung by glucocorticoids (Engineer *et al.*, 1978). These results can now be explained by the steroid-induced synthesis and release of macrocortin which inhibits phospholipase A_2 and the subsequent release of TxA_2 and prostaglandins (Blackwell, Carnuccio, Di Rosa, Flower, Parente & Persico, 1980), therefore suggesting that SRS-A and the leukotrienes stimulate phospholipase A_2.

Results obtained using LTC_4 and LTD_4 in isolated perfused lungs and parenchymal strips show these leukotrienes to have very similar actions and relative

potencies. They are approximately equipotent in releasing TxA_2 from isolated perfused lung and contracting parenchymal strips. This can be explained by the presence in unsensitized guinea-pig lung tissue of high levels of γ-glutamyltranspeptidase which are sufficient to convert tens of nmol/min of LTC_4 into LTD_4 (Morris, Taylor, Jones, Piper, Samhoun & Tippins, 1982).

As previously described for indomethacin (Piper & Samhoun, 1981), imidazole, a thromboxane synthetase inhibitor, greatly reduces by the same extent release of TxA_2 induced by either LTC_4 or LTD_4 from guinea-pig isolated perfused lung. Using radioimmunoassay of mono-*O*-methyl-TxB_2, other workers have also demonstrated the release of TxA_2 from guinea-pig perfused lung by LTC_4 and shown it to be antagonized by FPL 55712 (Berti, Folco & Omini, 1981).

Contractions of parenchymal strips elicited by LTB_4, LTC_4 and LTD_4 are also greatly inhibited by imidazole and carboxyheptylimidazole which is a very potent and specific inhibitor of thromboxane synthetase (Lewis & Watts, 1982). In the guinea-pig, parenchymal strips (*in vitro* preparations of peripheral airways) are more sensitive to LTC_4 and LTD_4 than larger airways such as the isolated trachea (Piper *et al.*, 1981) which could be due to thromboxanes being mainly synthesized in the parenchyma while the tracheal tissue produces mainly prostaglandin-like materials (Gryglewski, Dembinska-Kiec, Grodzinska & Panczenko, 1976). These results suggest that LTB_4, LTC_4 and LTD_4 exert their action in parenchymal strips mainly via generation of the potent bronchoconstrictor, TxA_2. However, there are differences between LTB_4 and leukotrienes C_4 and D_4 in the biological systems described in this paper. Unlike the peptidolipid leukotrienes, LTB_4 does not contract guinea-pig ileum, which suggests that the amino-acid residue at C-6 is a prerequisite for contraction of this tissue.

In parenchymal strips, tachyphylaxis occurs after repeated administration of LTB_4 but not after LTC_4 or LTD_4. Furthermore, FPL 55712, the SRS-A antagonist, has no effect on contractions of parenchymal strips induced by LTB_4 (as in the case of Bk) but antagonizes those due to LTC_4 and LTD_4. In conclusion, the results suggest that at the doses used LTB_4, LTC_4 and LTD_4 stimulate AA metabolism leading to generation of TxA_2 in guinea-pig lung *in vitro* but that LTB_4 acts on different receptors from those activated by the peptidolipid leukotrienes.

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