Br. J. Pharmac. (1985), **86**, 55–62

Differential release of eicosanoids by bradykinin, arachidonic acid and calcium ionophore A23187 in guinea-pig isolated perfused lung

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1 The effects of infusions of bradykinin (0.2 μM), calcium ionophore A23187 (0.5 μM) and arachidonic acid (13 μM) on the release of eicosanoids from the guinea-pig isolated perfused lung were investigated using radioimmunoassay for thromboxane B_2 (TXB_2), 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$), PGE_2, leukotriene B_4 (LTB_4) and LTC_4 and bioassay using the superfusion cascade.

2 Bradykinin released more 6-oxo-$PGF_{1\alpha}$ than TXB_2, whereas arachidonic acid and ionophore released more TXB_2 than 6-oxo-$PGF_{1\alpha}$.

3 The time course of eicosanoid release varied with the stimulus: bradykinin and arachidonic acid produced an immediate release, whereas the ionophore showed a slower onset of release.

4 Although the amounts of LTB_4 and LTC_4 released by the ionophore were very low according to radioimmunoassays, there was evidence from the bioassay of release of a leukotriene-like substance, thought to be LTD_4.

5 The leukotriene antagonist FPL 55712 lacks specificity in the guinea-pig trachea; at the concentration used (2 μM) it antagonized contractions of the tracheal strip to PGE_2 as well as to LTC_4.

6 Our results show that in the guinea-pig perfused lung the metabolism of exogenous arachidonic acid is both qualitatively and quantitatively different from the metabolism of endogenous arachidonic acid; furthermore, the profile of eicosanoid production is stimulus-dependent.

Introduction

Infusions or injections of arachidonic acid (AA) into the pulmonary circulation of guinea-pig isolated lungs lead to the formation of rabbit aorta contracting substance (RCS) (Piper & Vane, 1969), subsequently shown to be mainly composed of thromboxane A_2 (TXA_2) (Hamberg *et al.*, 1976). The metabolism of endogenous arachidonic acid leading to the synthesis of eicosanoids can also be triggered in guinea-pig lung by other stimuli, such as bradykinin and the calcium ionophore A23187 (Palmer *et al.*, 1973; Al-Ubaidi & Bakhle, 1980).

There is evidence, however, from other tissues (e.g. kidney, heart) that the eicosanoids formed from the metabolism of exogenous AA are different from those derived from endogenous AA released by bradykinin (Isakson *et al.*, 1976; Needleman *et al.*, 1979). We undertook this study to determine whether or not AA metabolism in guinea-pig lung was similarly dependent on the stimulus utilized.

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Methods

Preparation of the lungs

Male guinea-pigs (300–400 g body weight) were anaesthetized with pentobarbitone (60 mg kg^{-1} i.p.). Following mid-thoracotomy the pulmonary artery was cannulated and perfused for 10 min with 50 ml of heparinized Krebs bicarbonate solution (10 u ml^{-1}). The trachea was cannulated and the lungs removed and suspended in a heated chamber. The lungs were perfused via the pulmonary artery with warmed (37°C) and gassed (95% O_2–5% CO_2) Krebs bicarbonate solution at a constant rate of 5 ml min^{-1} (Bakhle *et al.*, 1969).

Collection of lung effluent

Arachidonic acid, bradykinin and A23187 were given either in 5 min infusions at 0.1 ml min^{-1} or as a single bolus injection. For radioimmunoassay (RIA) of eicosanoids, the lung effluent was collected in one minute fractions for the times shown (normally 15 min). In some experiments, a single collection over 3 or 4 min was made. In the experiments involving RIA, the lungs were used for a single infusion of a single stimulus (AA, bradykinin or A23187). When bioassay was used to identify eicosanoids, bolus injections of the stimuli were employed so that more responses could be obtained within the experimental lifetime of the lung (about 60 min).

Bioassay for leukotriene-like substance

Lung effluent was used to superfuse a series of bioassay tissues in a cascade (Vane, 1964): rat stomach strip (RSS), rabbit aorta (RbA), guinea-pig trachea (GPT) and guinea-pig ileum smooth muscle (GPISM). The bioassay tissues were arranged in two banks separated by a delay coil, such that the superfusing effluent after passing over the first bank of tissues was delayed by 2 min before passing over the second bank (Alabaster & Hawkeswood, 1978). The selectivity of the assay tissues was increased by an infusion into the lung effluent of a mixture of antagonists to histamine (mepyramine, 1 μM), acetylcholine (atropine, 1.5 μM), catecholamines (phenoxybenzamine, 0.3 μM and propranolol, 7 μM) and 5-hydroxytryptamine (methysergide bimaleate, 0.3 μM), (Gilmore *et al.*, 1968) together with indomethacin (5.6 μM) to prevent endogenous synthesis of prostaglandins by the assay tissues (Eckenfels & Vane, 1972).

Responses of the tissues were detected with auxotonic levers (Paton, 1957) attached to Harvard heart smooth muscle transducers and displayed on a Watanabe multichannel pen recorder.

The bioassay was used particularly to identify leukotriene-like substance (LT-LS) in the eicosanoid mixture in lung effluent. This identification was based on the following criteria. (i) The LT-LS would contract the GPT before and after the delay. (ii) No inhibition of its production occurred when indomethacin was infused through the lung. (iii) Inhibition of the GPT contraction to the LT-LS occurred after infusion of the leukotriene antagonist FPL 55712 over the assay tissues (Augstein *et al.*, 1973).

Radioimmunoassay of the lung effluent

The concentrations of TXB_2, 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$), PGE_2, LTB_4 and LTC_4 in the lung effluent were determined by specific radioimmunoassay (RIA) after suitable dilutions (1:2–1:100) in RIA buffer but without prior extraction or purification. The specificity of the antisera used in these RIAs has been previously established: TXB_2, 6-oxo-$PGF_{1\alpha}$ and PGE_2 (Salmon, 1978), LTB_4 (Salmon *et al.*, 1982) and LTC_4 (Aehringhaus *et al.*, 1982).

Materials

The Krebs bicarbonate solution had the following composition (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$, $7H_2O$ 1.17, $CaCl_2$, $6H_2O$ 0.25, $NaHCO_3$ 25 and glucose 8.4. Calcium ionophore A23187 (Calbiochem, La Jolla U.S.A.) was initially dissolved in absolute ethanol and then diluted in Krebs soution. Bradykinin triacetate salt (Sigma Chemical Company, Poole) was dissolved in distilled water. Arachidonic acid (Sigma) was initially dissolved in hexane, evaporated under N_2 and resuspended in 0.5 M methanolic NaOH to give a concentration of 10 mg AA ml^{-1}. The methanol was removed under N_2 and the residue resuspended in 1 M Tris buffer pH 9, then further diluted in Krebs solution. Indomethacin (Merck, Sharp & Dohme Ltd, Hertfordshire) was dissolved in 5% $NaHCO_3$. Other drugs used were:

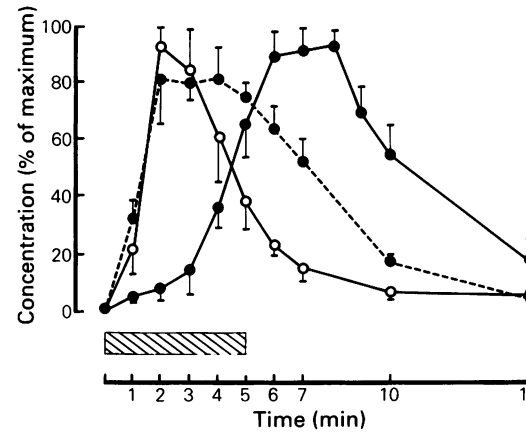

Figure 1 Time course of thromboxane B_2 (TXB_2) production from guinea-pig perfused lungs induced by A23187 (0.5 μM, ●———●), bradykinin (0.2 μM, ○———○) and arachidonic acid (AA; 13 μM, ●- - - -●). The points are the mean of data generated in 4 lungs and the vertical lines show the s.e.mean. The concentration of TXB_2 in each 1 min fraction of lung effluent was measured by radioimmunoassay. Since there were marked variations in the actual concentrations of TXB_2 released by different stimuli (see Figure 3), the data are presented as the percentage of the maximum concentration of TXB_2 released by each stimulus. The hatched rectangle shows the duration of the infusion of the agonist. The time course of TXB_2 production for each stimulus was very different, either in time of onset (compare AA and bradykinin with ionophore) or in duration (compare AA and ionophore with bradykinin).

phenoxybenzamine hydrochloride (Dibenyline; SK & F Stevenage), methysergide bimaleate (Sandoz Prods. Ltd, Leeds) mepyramine maleate (May & Baker, Dagenham), propranolol hydrochloride and atropine sulphate (Sigma). FPL 55712 (sodium 7-(3(4-acetyl-3-hydroxy-2-propylphenoxy)- 2-hydroxy-propoxy)-4-oxo-8-propyl- 4H-1-benzopyran- 2-carboxylate) was a gift from Fisons Ltd, Loughborough; U-46619 (11α,9α-epoxymethano prostaglandin H_2) and PGE_2 were gifts from the Upjohn Co., Kalamazoo, U.S.A. Antiserum against LTC_4 was kindly provided by Dr B. Peskar (University of Bochum, West Germany). 5,6,8,9,11,12,14,15 (n)-[^{3}H]-TXB_2, specific activity, (s.a.) 140 Ci mmol^{-1}; 5,6,8,11,12,14,15(n)-[^{3}H]-PGE_2, s.a. 160 Ci mmol^{-1}; 6-oxo-5,6,8,9,11,14,15,(n)-[^{3}H]-$PGF_{1\alpha}$, s.a. 150 Ci mmol^{-1} and 5,6,8,9,11,12, 14,15(n)-[^{3}H]-LTB_4, s.a. 221 Ci mmol^{-1} were purchased from Amersham International (Amersham, Bucks). 14,15(n)-[^{3}H]-LTC_4, s.a. 34 Ci mmol^{-1} was obtained from New England Nuclear (Boston, Mass., U.S.A.).

Statistics

Results are shown as mean values ± s.e.mean for n experiments. Student's unpaired t test was used to determine the significance of differences between means and a P value of <0.05 was taken as significant.

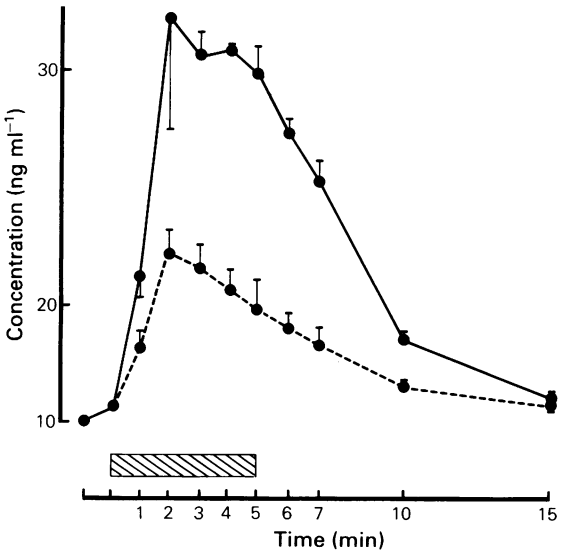

Figure 2 Time course of thromboxane B_2 (TXB_2, ●———●) and 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$, ●- - - -●) production from guinea-pig perfused lungs induced by arachidonic acid (13 μM). The points show the mean concentrations of TXB_2 and 6-oxo-$PGF_{1\alpha}$ from 4 lungs and the vertical lines show the s.e.mean. The hatched rectangle shows the period of the infusion. These experiments were carried out as in Figure 1. Note that absolute concentrations of eicosanoid are shown and that about three times as much TXB_2 as 6-oxo-$PGF_{1\alpha}$ is produced.

Results

Time-course of eicosanoid release

Infusion of arachidonic acid (13 μM) into the guinea-pig lung led to the synthesis of TXA_2 (measured as TXB_2 by RIA). This synthesis reached a plateau in the second minute and was maintained until the end of the infusion, falling immediately after the infusion was finished (Figure 1). Bradykinin (0.2 μM) induced synthesis of TXB_2 which peaked during the first 2 min and declined thereafter. The response to A23187 (0.5 μM) was, however, different from either of the first two agonists. The release slowly increased during the infusion period and only reached a maximum after the end of the infusion. The fall in TXB_2 production was also slow; by 10 min, TXB_2 levels were about half the maximal value and at 15 min TXB_2 levels were still higher than before stimulation. Measurements of 6-oxo-$PGF_{1\alpha}$ in the same lung effluent samples showed that the production of this eicosanoid had the same time course as that of TXB_2 for all three agonists. This is illustrated in Figure 2 in response to AA infusion. The time course for PGE_2, LTB_4 and LTC_4 (the last two were detectable only when using the calcium ionophore A23187) was similar to that of TXB_2 (data not shown).

Ratios of eicosanoids formed

At the concentrations shown in Figure 3, all three stimuli (AA, bradykinin and the ionophore) produced approximately equal concentrations of prostacyclin (PGI_2) measured as immunoreactive 6-oxo-$PGF_{1\alpha}$. The samples were taken at the time of maximum eicosanoid production. For infusion of AA, the effluent samples were taken from the second to the fifth minute; for bradykinin from the second to the fourth minute and for A23187 from the sixth to the ninth minute. Bradykinin released more 6-oxo-$PGF_{1\alpha}$ than TXB_2, whereas arachidonic acid and A23187 released more TXB_2 than 6-oxo-$PGF_{1\alpha}$. The ionophore was the most potent stimulus for releasing TXB_2. Levels of PGE_2 were low in comparison to those of TXB_2 and 6-oxo-$PGF_{1\alpha}$ and the levels of LTB_4 and LTC_4 were much lower still (less than 1 ng ml^{-1}) and are not shown. The ratio between TXB_2 and 6-oxo-$PGF_{1\alpha}$ did not change qualitatively by varying the dose of any of the three stimuli but increased when the dose of AA was augmented (Table 1).

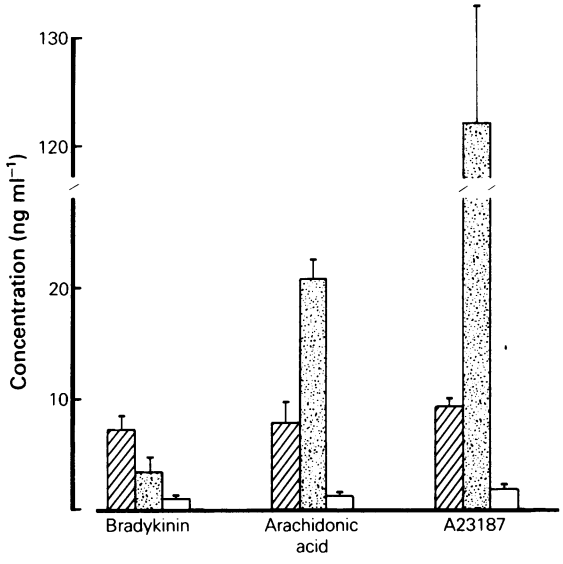

Figure 3 Release of 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$; hatched columns), thromboxane B_2 (TXB_2; stippled columns) and PGE_2 (open columns) from guinea-pig perfused lungs induced by infusion of bradykinin (0.2 μM), arachidonic acid (13 μM) and the ionophore A23187 (0.1 μM). Each column represents the mean of 5 experiments and the vertical lines show the s.e.mean. The values shown are the concentrations found in single samples of lung effluent taken during the time of maximum production of eicosanoid (see text). Whereas the release of PGI_2 (as 6-oxo-$PGF_{1\alpha}$) was approximately the same for all three stimuli, release of TXA_2 (measured as immunoreactive TXB_2) was markedly different for each stimulus.

Table 1 The ratio of thromboxane B_2 (TXB_2) to 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) produced from guinea-pig isolated lung: effect of different concentrations of stimulant agonists

Stimulus	*Concentration* (μM)	*Ratio*
Bradykinin	0.2	0.4 + 0.1
Bradykinin	2	0.4 + 0.1
A23187	0.1	8.5 + 0.6*
A23187	0.5	10.9 + 1.0
Arachidonic acid	13	3.1 + 0.8
Arachidonic acid	65	9.4 + 1.2*

The ratios shown are the mean values (± s.e.mean) from 5 experiments (lungs) at each concentration for each stimulus. Only one stimulus was given to each lung. Eicosanoids in lung effluent were measured by radioimmunoassay as described for Figure 3.
*Significant $P < 0.05$.

Bioassay of eicosanoids

Using the same concentrations of agonist (AA, 13 μM; bradykinin, 0.2 μM and A23187, 0.5 μM), we also analysed the eicosanoid production by bioassay, using the two banks of assay tissues separated by a delay coil (see Methods). Following the standard 5 min infusion of each of the agonists, contraction of GPT before and after the delay was observed, although the immunoreactive LTC_4 was always very low (see above). Because of the discrepancy between RIA and bioassay for LT-LS, we examined the nature of the LT-LS further, but using injections rather than infusions of agonist. This enabled more responses to be obtained within the experimental life time of the isolated lung preparation.

Injection of AA into the lung caused the release of substances that contracted the RSS, GPT and RbA as illustrated by the experiment shown in Figure 4. Responses of the GPT and RSS in the second bank of tissues after the delay were still present but that of the RbA had virtually disappeared. The next set of tissue responses followed injection of the ionophore A23187 through the lung and showed a qualitatively similar pattern with contractions of the GPT and RSS before and after the delay. The last two sets of responses demonstrate that even after the delay the RbA was capable of contracting to a stable agonist, the endoperoxide analogue U46619, and that authentic LTC_4 contracted the GPT and RSS before and after the delay but was without effect on the RbA. These suggestions of the presence of a LT-LS in lung effluent were further tested by using indomethacin.

In the experiment illustrated in Figure 5, the initial responses to stimulation of the lung with AA were abolished by infusing indomethacin through the lung. However, during this infusion the ionophore was still able to release from lung a material contracting the GPT before and after the delay, although the RbA contracting material was not apparent. Injections of bradykinin through the lung released biological activity comparable to that following AA, and as with AA, all activity was absent after indomethacin treatment of the lung.

The third criterion of LT-LS, antagonism by FPL 55712, was then applied. In Figure 6 the responses of two GPTs, both after the delay, are shown. The first response on both tissues was to authentic LTC_4 and then an infusion of FPL 55712 was started only over the lower GPT. This prevented its response to a larger amount of LTC_4 and to stimulation by AA through the lung, although the upper GPT contracted to both injections. The last injection was of the ionophore and here too the upper GPT contracted but the lower GPT was blocked by the FPL 55712 infusion. Results similar to those shown here for AA stimulation were obtained with bradykinin stimula-

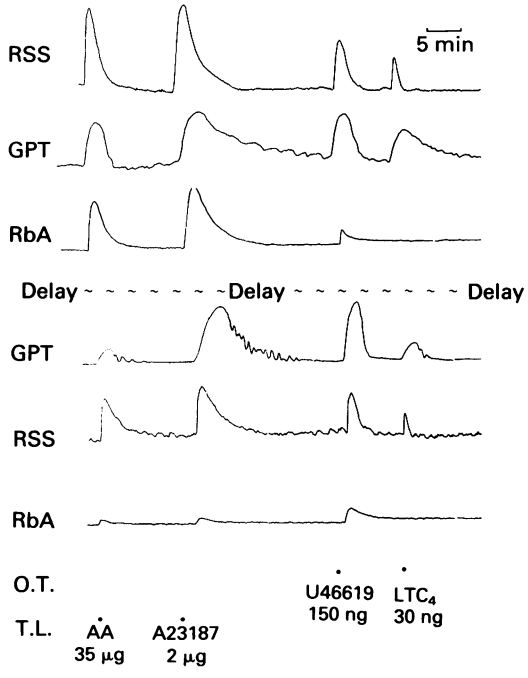

Figure 4 Bioassay of eicosanoids produced from guinea-pig lung following arachidonic acid (AA) or ionophore injections into the pulmonary circulation. Perfusate effluent from the isolated lung superfused two banks of assay tissues separated by a 2 min delay coil (marked Delay on the figure). The first set of responses was to AA (35 μg) injection; note the loss of rabbit aorta (RbA)-contracting activity after the delay and that the guinea-pig trachea (GPT) contracting activity survived although decreased relative to rabbit stomach strip (RSS) contracting activity. With the ionophore (2 μg), all the contractions in both banks were larger than those to AA, but the RbA-contracting activity was still lost while the GPT contracting activity still survived. The third set of responses to U46619 (150 ng), a stable thromboxane A_2 (TXA_2)-mimetic, shows that both RbA tissues were capable of responding and that the lack of contraction of the second RbA (after the delay) in the previous sets of responses was due to the degradation to TXA_2 in the delay coil. Comparison of the last set of responses, to authentic leukotriene C_4 (LTC_4; 30 ng), with those to AA and ionophore given through the lung suggests that a leukotriene-like substance was produced by either stimulus in addition to TXA_2. The assay tissues did not respond to either AA or ionophore given directly to the tissues (not shown). The same pattern of responses was observed in another four experiments. T.L. = injection through the lung; O.T. = injection over tissues.

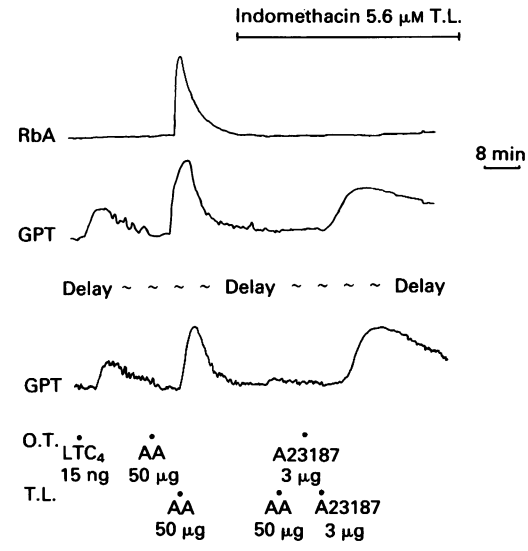

Figure 5 Effect of indomethacin on leukotriene-like substance (LT-LS) release by arachidonic acid (AA) and ionophore. In this experiment, the LT-LS generated by AA (50 μg) given through the lung was absent after indomethacin treatment of the lung (5.6 μM), but the guinea-pig trachea (GPT) contracting activity stimulated by ionophore (3 μg) was still produced. The first contraction of the GPT was induced by an injection of authentic LTC_4 (15 ng) given directly to the tissues. The same pattern of responses was observed in another four experiments. T.L. = injection through the lung; O.T. = injection over tissues; RbA = rabbit aorta.

tion of guinea-pig lung, indicating release of a LT-LS.

The results so far suggest that although the LT-LS produced by ionophore met all three criteria, that produced by AA or bradykinin behaved like a cyclo-oxygenase product but was still antagonized by FPL 55712. We therefore examined the specificity of FPL 55712 on the GPT.

Two isolated smooth muscle strips, GPT and GPISM, were superfused with Krebs solution containing indomethacin (5.6 μM) and their responses to authentic LTC_4 and PGE_2 observed. One of four experiments is illustrated in Figure 7; the GPT and GPISM contracted to both LTC_4 and PGE_2. After treatment with FPL 55712 (2μM) the GPISM no longer responded to LTC_4 but still contracted to PGE_2 showing the expected specificity of antagonism. However, on the GPT, contractions to both LTC_4 and PGE_2 were abolished by this antagonist. At lower concentrations (0.5 μM−1 μM), FPL 55712 neither abolished LTC_4 contractions nor differentiated between LTC_4 and PGE_2 on the GPT (data not shown).

At this stage, it appeared that there were considerable amounts of bioassayable LT-LS released from lung by ionophore stimulation either by injection or infusions. We had already found very low levels of immunoreactive LTC_4 (and LTB_4) in the effluent following infusions of ionophore A23187 (see above) and to confirm the discrepancy between RIA and

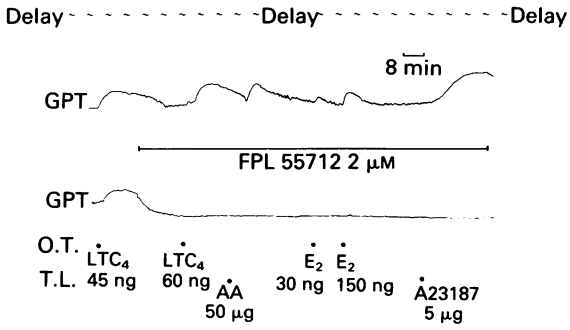

Figure 6 Effect of FPL 55712, a leukotriene antagonist on the leukotriene-like substance (LT-LS) produced from guinea-pig lung stimulated by injections of arachidonic acid (AA) and A23187. After the first injection of authentic LTC_4 (45 ng) directly to the assay tissues, the infusion of FPL 55712 (2 μM) over the lower guinea-pig trachea (GPT) was started and this infusion prevented the lower tissue from responding to the second, larger, dose of LTC_4 (60 ng). Injections of AA (50 μg) or of ionophore (5 μg) through the lung caused contractions of the upper untreated GPT whereas the lower FPL-treated GPT remained unresponsive. The same pattern of responses was observed in four other experiments. T.L. = injection through the lung; O.T. = injection over tissues; E_2 = prostaglandin E_2.

bioassay for injections of ionophore, a combined bioassay and RIA experiment was performed. Only one GPT was superfused with lung effluent and the superfusate was collected in 1 min fractions immediately below the assay tissue. Collections were

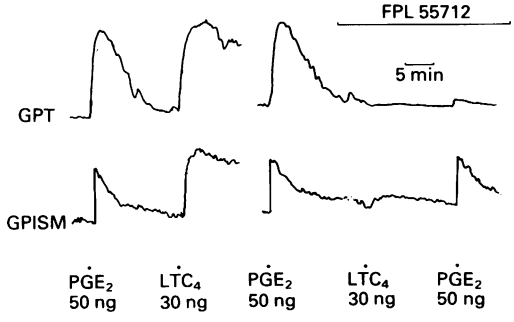

Figure 7 Effect of FPL 55712 (2 μM) on the contractions induced by prostaglandin E_2 (PGE_2; 50 ng) and leukotriene C_4 (LTC_4; 30 ng) on guinea-pig trachea (GPT) and guinea-pig ileum longitudinal smooth muscle (GPISM) preparations. GPT and GPISM strips were superfused with Krebs solution containing indomethacin (5.6 μM). The first and third contraction were induced by standard doses of PGE_2 (50 ng). The second contraction was induced by a known amount of LTC_4 (30 ng). The same pattern of responses was observed in four other experiments.

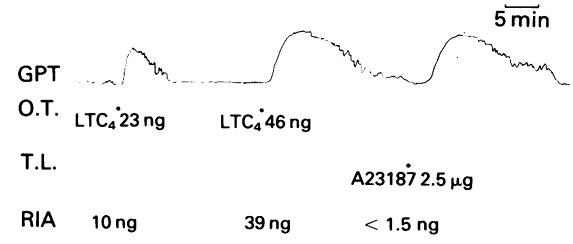

Figure 8 Lack of correlation of measurement of leukotriene C_4 (LTC_4) by radioimmunoassay with bioassay estimates of leukotriene-like substance (LT-LS) produced by guinea-pig isolated lung. Effluent from lung perfused with Krebs solution containing indomethacin (5.6 μM) superfused a single guinea-pig trachea (GPT). The first two contractions were induced by known amounts of LTC_4 (23 ng and 46 ng respectively) given directly over the assay tissues (O.T.) and the third contraction followed an injection of A23187 (2.5 μg) into the lung (T.L.). On the bottom the amounts of immunoreactive LTC_4 (10 ng and 39 ng respectively) recovered by radioimmunoassay of the effluent are shown.

started 2 min before injections and continued until just after the peak of the assay tissue response. The fractions were then assayed by RIA for LTC_4. As shown in Figure 8, the first two tissue responses are to direct injections of LTC_4 (23 and 46 ng) and below the experimental trace is shown the amount of LTC_4 recovered (10 and 39 ng, respectively). The third response was to ionophore injection through an indomethacin-treated lung and, although the response was clearly greater than that to 23 ng and almost as large as that to 46 ng, less than 1.5 ng LTC_4 was detected by RIA. Thus, either with injections or infusions the majority of the bioassayable LT-LS does not appear to be immunoreactive LTC_4.

Discussion

Our experiments show that the eicosanoid metabolites of AA that are found in the effluent from guinea-pig isolated lung vary with the nature of the stimulus. Differences between exogenous and endogenous metabolism of AA have already been described, using bradykinin and AA stimulation in other isolated organs (Isakson *et al.*, 1976; Needleman *et al.*, 1979). We show here in guinea-pig isolated lung that qualitative differences can be readily demonstrated in the TXB_2:6-oxo-$PGF_{1\alpha}$ ratio; for instance, bradykinin was the only stimulus to yield more 6-oxo-$PGF_{1\alpha}$ than TXB_2 and both the other two stimuli favoured TXB_2 release. The balance between 6-oxo-$PGF_{1\alpha}$ and TXB_2 is not simply related to endogenous vs. exogenous substrate as the two extremes of our range of stimuli (bradykinin and ionophore) both induce eicosanoid

production from endogenous AA. The apparent inefficiency of cyclo-oxygenase in guinea-pig lung in metabolizing exogenous AA (only 2% of the AA infused was converted into TXB_2, 6-oxo-$PGF_{1\alpha}$ and PGE_2) is commonly observed (Al-Ubaidi & Bakhle, 1980; Jose & Seale, 1979) and may be due to competition for the exogenous substrate between cyclo-oxygenase and incorporation into lipids.

It has been suggested that PGI_2 is released mainly by the vasculature (endothelial cells) and TXA_2 by the parenchymal cells in lung (Gryglewski *et al.*,1978). Bradykinin has already been shown to increase preferentially 6-oxo-$PGF_{1\alpha}$ synthesis in relation to that of TXB_2 in lung and vascular tissues of several species (Saldeen & Saldeen, 1983), although in the lung specimens used by these authors the pulmonary vessels could have been the source of both eicosanoids. Furthermore, in cultured endothelial cells, the ratio of 6-oxo-$PGF_{1\alpha}$ to TXB_2 was always in favour of the former whether basal or stimulated formation of eicosanoids was measured (Ingerman-Wojenski *et al.*, 1981; Crutchley *et al.*, 1983). In rabbit pulmonary artery, the ratio is almost identical with that found here (Salzman *et al.*, 1980). Thus, it is likely that the activity of bradykinin in our preparation is on the pulmonary vascular endothelial cells. It is interesting that in isolated perfused kidney, bradykinin favoured the synthesis of PGE_2 whereas AA favoured that of PGI_2 (Needleman *et al.*, 1979).

The response to the calcium ionophore A23187 exhibited two interesting characteristics – the delay in the formation of eicosanoids and the presence of a bioactive LT-LS, probably LTD_4. The delay may represent the stimulation of a population of cells physically distant from the pulmonary vessels or one with relatively difficult access, for instance, cells in the major airways further from the alveoli. In this instance, there would be a delay both in the stimulus arriving at the cells and in the eicosanoids formed diffusing to the pulmonary vessels and appearing in lung effluent. Alternatively, the delay could be due to the several steps (for instance, enzymic reactions or cell–cell interactions) involved in the ionophore-stimulated eicosanoid production.

In relation to the release of a leukotriene-like substance, our criteria for identifying it as LT-LS were all met, but the responses of the bioassay tissues were greatly in excess of the immunoreactive LTC_4 measured in effluents. The LT-LS could therefore be either LTD_4 or LTE_4, which are both capable of contracting the assay tissues, although their potency is different (Lewis *et al.*, 1981). Since LTC_4 is rapidly metabolized to LTD_4 by guinea-pig lung homogenates (Sirois & Brousseau, 1983) and LTD_4 is the major component of guinea-pig slow-reacting substance of anaphylaxis (SRS-A; Morris *et al.*, 1982), in our experiments the LT-LS is most probably LTD_4. It is relevant to note that synthesis of SRS-A (probably a mixture of LTC_4 and LTD_4) by lung in response to ionophore stimulation has already been demonstrated (Piper & Seale, 1979).

It is interesting that the other two stimuli (AA and bradykinin) did not cause the formation of a leukotriene-like substance, since in the lung parenchymal strip of the guinea-pig, incubation with AA leads to the formation of LTC_4 and LTD_4 (Burka & Saad, 1984). This discrepancy may be due to access to a different range of cell types in the two preparations or to differences in substrate availability or enzyme activation.

Our experiments have also illustrated very clearly that the leukotriene antagonist FPL 55712 lacks specificity on the GPT in so far as it also inhibits contractions due to PGE_2. The specificity of this antagonist on the tissue originally used, the GPISM (Augstein *et al.*, 1973), was confirmed. Welton *et al.* (1981), showed that FPL 55712, at higher concentrations (2–10 μM), also inhibits thromboxane synthase in human platelet microsomes and antigen-induced histamine release from actively sensitized guinea-pig lung fragments. The more general inference from these results is that the specificity of antagonists should not be assumed for tissues other than those on which it has been demonstrated. Furthermore, contractions of the GPT reversible by FPL 55712 cannot be taken in isolation as evidence of leukotriene production.

The measurement of PGI_2 and TXA_2 synthesis in the lung by monitoring the release of their stable hydrolysis products, 6-oxo-$PGF_{1\alpha}$ and TXB_2 respectively, underestimates their total synthesis since both products can be metabolized by the lung (Dawson *et al.*, 1976). The degree of metabolism apparently varies if the lung is stimulated with different agents (Boot *et al.*, 1978; Robinson *et al.*, 1984) and consequently it is impossible to extrapolate from our data to total synthesis of either PGI_2 or TXA_2. However, measurement of 6-oxo-$PGF_{1\alpha}$ and TXB_2 probably provides a valid assessment of the biologically active prostacyclin and TXA_2 in the perfusate, since it does correlate with the bioassay data.

Overall, our results suggest that the profile of eicosanoid production in an intact tissue is not only species-dependent (Al-Ubaidi & Bakhle, 1980; Ally *et al.*, 1980) and cell-dependent (Salmon & Flower, 1979), but is also stimulus-dependent. In lung the stimulus-dependent response probably reflects the different populations of cells excited by each stimulus and, by extension, the different mixture of lipoxygenase, cyclo-oxygenase, PGI_2 and TXA_2 synthases in those populations. The finding that bradykinin produced more 6-oxo-$PGF_{1\alpha}$ than TXB_2 may reflect a selective stimulation of the vascular compartment whereas the release induced by the ionophore A23187 indicates an effect on other cell type compartments.

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(Received December 6, 1984.
Revised April 25, 1985.
Accepted May 2, 1985.