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Release of arachidonic acid metabolites and histamine from sensitized guinea-pig lung following antigen challenge

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1 The time course of mediator release and the hypothesis that the ratio of eicosanoids to histamine might alter with the intensity of stimulus or its route of administration has been explored in isolated perfused lung from sensitized guinea-pigs challenged with ovalbumin.

2 Histamine and prostaglandin release was rapid in onset and virtually complete within 10 min. Thromboxane B_2 (TXB_2) and leukotriene D_4 (LTD_4) release, however, was more sustained. Release of the major prostanoid metabolites was relatively delayed compared to that of the parent compounds and was more sustained.

3 Mediator release was antigen-dose dependent and TXB_2, prostaglandin D_2 (PGD_2) and LTD_4 release linearly related to histamine concentrations ($P < 0.05$). However, the ratio of the percentage maximum release of eicosanoids relative to histamine was greatest with low doses of ovalbumin.

4 At a low antigen dose (10 μg ovalbumin), histamine and prostanoid release was greatest when the challenge was via the airway rather than into the pulmonary artery and the greatest differences were in $PGF_{2\alpha}$ levels. At near maximal challenge (1 mg ovalbumin) there was little difference in concentrations of PGD_2, TXB_2, 6-oxo-$PGF_{1\alpha}$ and LTD_4 by either route, but $PGF_{2\alpha}$ levels remained greater.

5 The results indicate that biologically active amounts of prostanoids may be released from sensitized lung at low degrees of mast cell activation and that differences in mediator release following antigen administration to the airway or into the pulmonary vasculature simply reflects its accessibility to sensitized cells.

Introduction

The pathological features of type I hypersensitivity reactions in the lung result from the combined actions of pre-formed mediators and newly formed lipid products released from sensitized cells as a consequence of the interaction between reaginic antibody and antigen (Kagey-Sobotka *et al.*, 1982; Ogunbiye & Eyre, 1985). During anaphylaxis in animal and human lung *in vitro*, histamine and a number of metabolites of arachidonic acid such as prostaglandins, thromboxanes and leukotrienes are released (Piper & Vane, 1969; Schulman *et al.*, 1981). Pharmacological studies have implicated histamine and the peptidoleukotrienes as the major mediators of antigen-induced contractions of bronchial smooth muscle *in vivo* and *in vitro* (Andersson, 1982; Burka, 1985a, b; Adams & Lichtenstein, 1985), however, there seems to be some uncertainty over the biological significance of cyclo-oxygenase products. Perfusion of isolated guinea-pig lung with exogenous histamine, slow reacting substance of anaphylaxis (SRS-A) or purified leukotrienes C_4 or D_4 results in the stimulation of prostaglandin and thromboxane efflux (Engineer *et al.*, 1978; Berti *et al.*, 1979; Piper & Samhoun, 1982) suggesting that some prostanoid release in anaphylaxis might be a secondary or indirect event.

We have investigated the time-course of release of histamine, leukotriene D_4 (LTD_4) and cyclo-oxygenase products from guinea-pig isolated lungs in an attempt to determine whether release of some eicosanoids might be dependent on the prior release of other inflammatory mediators. Furthermore, eicosanoid release from guinea-pig lung has been reported to be stimulus-dependent (Bakhle *et al.*, 1985a,b). We have explored the possibility that their release may also vary with the intensity of stimulus or its route of administration.

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Methods

Sensitizations

Male Dunkin-Hartley guinea-pigs (300–500 g) were sensitized with 100 mg ovalbumin in 1 ml 0.9% saline divided i.p. and s.c. A booster dose of 50 mg ovalbumin was injected i.p. 3 days later and they were studied after 21–28 days (Andersson, 1982). Sham-sensitized animals were similarly treated with saline alone.

Lung perfusion

Guinea-pigs were killed by cervical dislocation, the trachea cannulated and the lungs ventilated with humidified room air at a rate of 60 breaths min^{-1} at an end expiratory pressure, measured at the trachea, of 2 mmHg and an initial lung inflation pressure of 8–10 mmHg. The thorax was opened, the pulmonary artery cannulated (normally within 5 min of death) and the left atrium transected. The lungs were perfused with Krebs solution (mM composition: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.1) at 37°C gassed with 95% O_2 : 5% CO_2, at a rate of 10 ml min^{-1}. Preparations which showed visible surface damage were discarded. A bubble trap was used to prevent air emboli. The heart was cut away, the lungs removed and suspended by the tracheal cannula inside a jacketed chamber maintained at 37°C and covered at the top with moistened gauze. The lungs were left to stabilize for 30 min and then challenged with bolus injections of ovalbumin (1 μg–10 mg in 0.2 ml saline) directly into the perfusate flow. Ventilation was stopped immediately following challenge. Pulmonary artery perfusion pressure was measured with a Statham pressure transducer attached to a side arm from the pulmonary artery cannula and recorded on a Grass polygraph. Perfusate was collected for timed intervals pre- and post-challenge and immediately cooled to 4°C. Aliquots of perfusate were kept at −80°C until analysed for histamine and at −20°C until analysed for prostanoids and leukotrienes; 2 ng of deuterated prostaglandin E_2 (PGE_2), $PGF_{2\alpha}$, thromboxane B_2 (TXB_2), and 6-oxo-$PGF_{1\alpha}$ were added to the prostanoid samples to act as internal standards. [^{3}H]-LTD_4 was added to those samples for leukotriene estimations. For perfusion via the trachea the lungs were initially prepared as described above and perfused for 10 min via the pulmonary artery. During this period the lungs were inflated with 20 ml of air and light scarifications made, as uniformly as possible with a 27 gauge needle on the surfaces of the inflated lobes. The lungs were suspended inside the heated jacket, the pulmonary artery cannula removed and the perfusate flow connected to the tracheal cannula (10 ml min^{-1}). They were allowed to stabilise for 30 min before challenge with ovalbumin. The perfusion fluid escaped from the alveoli via the scarifications made in the lung surface.

Protocols

Only one challenge was performed on lungs from each animal. Experiments were carried out according to the following protocols:

(1) In the series of time course studies, 1 min collections of perfusate were made 10 min and 1 min before ovalbumin challenge, every minute for the first 5 min immediately following challenge and at 10, 15 and 30 min post-challenge. In these experiments challenge was with 200 μg ovalbumin in 0.2 ml 0.9% saline.

(2) In the dose-response studies, perfusate was collected for a 10 min period (a) immediately pre- and (b) post-challenge. The same collections were made whether perfusion and challenge was via the pulmonary artery or via the trachea.

Extraction and derivatisation of prostanoids

Prostanoids were extracted and derivatised by a modification of the method described by Waddell *et al.* (1984). Aliquots of lung perfusate with added internal deuterated standards were made up to 10 ml with equal volumes of distilled water and 3 M acetate buffer, pH 5.2. Oxo functions were converted to methoximines by incubation with 1 ml methoxyamine hydrochloride in water (100 mg ml^{-1}) at 60°C for 15 min. After cooling to room temperature the perfusate was adjusted to pH 3.5–4.0 with 3 M HCl and extracted into 7 ml ethyl acetate by use of reverse phase C_{18} Sep-Pak cartridges which had been preconditioned with 5 ml ethyl acetate, 5 ml methanol and 5 ml distilled water. The prostanoids eluting in the ethyl acetate phase were applied to a silica straight phase cartridge (preconditioned with 5 ml methanol and 5 ml ethyl acetate), eluted with 5 ml methanol and the solvent evaporated to dryness under nitrogen. Carboxyl groups were converted to pentafluorobenzyl esters by incubation for 15 min at room temperature with 35% pentafluorobenzyl bromide (PFBB) in acetonitrile, following resuspension in acetonitrile and N,N-diisopropylethylamine (3 : 1 v/v). Reagents were evaporated under nitrogen and hydroxyl functions converted to trimethylsilyl ethers by adding 100 μl *bis*-(trimethylsilyl)trifluoroacetamide (BSTFA) and allowing to stand overnight at room temperature.

The silylating reagent was evaporated under nitrogen and the residue dissolved in *n*-dodecane prior to

gas chromatography-negative ion chemical ionisation/mass spectrometry (gc-nici/ms).

Selected ion monitoring was performed on a Finnigan 4500 mass spectrometer using a 30 metre Chrompak Sil-5 gas chromatography column. Column temperature was programmed from 200°C to 260°C at 20°C min^{-1} and then from 260°C to 325°C at 3°C min^{-1} which allowed resolution of the monitored masses. Helium was used as the chromatography carrier gas at 1 ml min^{-1} and the capillary column pressure maintained at 20 psi. Ionizer temperature was 150°C, ionizer pressure 0.4 torr, emission current 0.3 A, electron energy 100 eV and ammonia used as the reagent gas.

Histamine assay

Histamine was measured spectrophotofluorimetrically following condensation with *o*-phthalaldehyde by the method of Håkanson *et al.* (1972).

Leukotriene assay

Analysis was carried out on samples, adjusted to pH 7, spiked with 2000 d.p.m. [^{3}H]-LTD_4 as internal standard and extracted in 7 ml methanol by use of C_{18} reverse phase Sep-Pak cartridges preconditioned with ethyl acetate, methanol and water. The methanol was evaporated under nitrogen and the residue resuspended in 300 μl radioimmunoassay (RIA) buffer. An aliquot was counted for estimation of recovery and the remainder used for RIA with rabbit anti-LTC_4 antibody, having a 71 $\pm$ 6% cross reactivity for LTD_4. Bound ligand was separated from free with activated charcoal/dextran solution. Standard curves were constructed for LTD_4 giving detection limits in the RIA of 40.7 $\pm$ 9 pg and 50% displacement of [^{3}H]-LTC_4 at 363 $\pm$ 15 pg. Mean overall recovery was 67 $\pm$ 1% ($n = 127$). Non-specific binding was determined in RIA buffer (Richmond *et al.*, 1987). LTD_4 concentrations in Krebs solution blanks after Sep Pak extraction were below the detection limits of the assay.

Drugs

The following were used: ovalbumin, indomethacin, NDGA, *n*-dodecane, BSTFA, N,N-diisopropylethylamine (Sigma Ltd., Poole, Dorset); methoxyamine hydrochloride (Aldrich Chemical Co., Gillingham, Dorset). PFBB (Fluorochem, Glossop, Derbyshire); LTD_4 (Merck-Frosst Canada Inc., Pointe Claire-Dorval, Canada); [^{3}H]-LTC_4 and [^{3}H]-LTD_4 (New England Nuclear, Hertfordshire); prostanoids and deuterated standards (the kind gift of Dr Pike, Upjohn Co., Kalamazoo, M.I., U.S.A.) and Sep-Paks (Waters Associates, Northwich, Cheshire).

Statistics

Data are presented as mean $\pm$ s.e.mean. Differences in mediator concentrations in airway or artery perfused lungs following antigen challenge were assessed by the Mann-Whitney U test. Correlations between mediator concentrations were determined by multiple linear regression.

Results

Eicosanoids and histamine were detected, in low levels, in perfusates from sham-sensitized lungs; TXB_2 and 6-oxo-$PGF_{1\alpha}$ were the major prostanoids present. In sensitized lung TXB_2 represented 60% of the total prostanoid released.

Ovalbumin challenge over the range 1 μg–10 mg was associated with a dose-related increase in histamine and eicosanoid concentrations in the perfusion fluid (Figure 1). The principal eicosanoid detected was TXB_2, which at maximal observed stimulation was increased a mean 30 fold over resting levels. PGD_2 concentrations increased a mean 48 fold and 6-oxo-$PGF_{1\alpha}$, 14 fold after challenge. $PGF_{2\alpha}$ and PGE_2 comprised only 4% of the total prostanoid released. In addition, there was an 82 fold increase in LTD_4 and a mean 26 fold increase in histamine concentrations after maximal challenge. Multiple linear regression showed significant linear correlations between histamine, PGD_2, LTD_4 and TXB_2 ($P < 0.05$) concentrations which with the exception of TXB_2 ($P < 0.001$) passed through the origin. The ratio of prostaglandin to histamine release, as a percentage of their respective maxima, was greater at low than at high antigen doses.

Figure 2 shows the time course of release of the mediators. Histamine release reached a peak within 1 min of challenge and was virtually complete by 10–15 min. The time-course of PGD_2, $PGF_{2\alpha}$, PGE_2 and 6-oxo-$PGF_{1\alpha}$ release were almost indistinguishable from that of histamine except that maximum rates of release were reached at 2 min post-challenge. Release of TXB_2 and LTD_4 into the perfusate was more sustained (Figure 2). At low provocation doses (10 μg ovalbumin) histamine, PGD_2, TXB_2, 6-oxo-$PGF_{1\alpha}$ and $PGF_{2\alpha}$ release was greater when the antigen was applied via the airway (Table 1). Whilst concentrations of all eicosanoids were elevated, the greatest differences were in $PGF_{2\alpha}$ levels. LTD_4 concentrations were similar, regardless of the route of antigen administration. At maximum or near-maximal challenge (1 mg ovalbumin) there was little difference in PGD_2, TXB_2, 6-oxo-$PGF_{1\alpha}$,

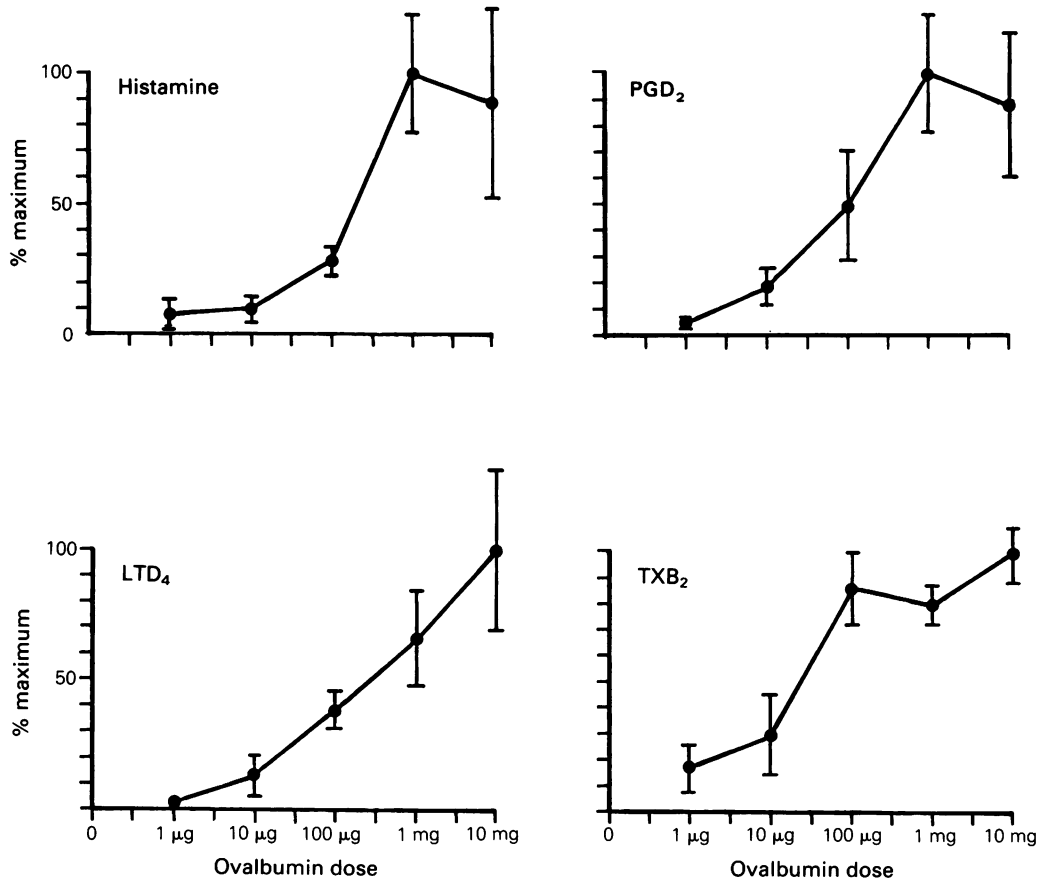

Figure 1 Increase in release of histamine, prostaglandin D_2 (PGD_2), leukotriene D_4 (LTD_4), and thromboxane B_2 (TXB_2) from sensitized guinea-pig isolated perfused lung following increasing ovalbumin challenge. The results are expressed as a % of maximum increases in concentration. Each point is the mean of 5 observations. Maximum concentrations were (ng ml^{-1}) histamine 240.8 ± 38.0, LTD_4 1.97 ± 0.62, PGD_2 10.1 ± 1.6, TXB_2 38.9 ± 4.9.

Table 1 Comparison of mediator concentrations released from guinea-pig lung perfused and challenge via the pulmonary artery or via the trachea

	10 μg Ovalbumin		*1 mg Ovalbumin*	
(ng ml^{-1})	*Artery*	*Airway*	*Artery*	*Airway*
Histamine	23.3 ± 13	85.0 ± 23.9*	235 ± 38	162 ± 44.5
LTD_4	0.26 ± 0.2	0.33 ± 0.04	1.27 ± 0.4	0.87 ± 0.26
PGD_2	1.77 ± 0.8	4.91 ± 0.98*	9.78 ± 1.57	7.63 ± 1.37
TXB_2	10.6 ± 6	33.1 ± 5.0*	28.8 ± 3.0	28.3 ± 1.9
6-oxo $PGF_{1\alpha}$	2.03 ± 1.3	5.96 ± 0.67*	3.96 ± 1.1	6.59 ± 1.69
$PGF_{2\alpha}$	0.16 ± 0.1	1.39 ± 0.25†	0.42 ± 0.2	2.66 ± 0.6†
PGE_2	0.21 ± 0.1	0.43 ± 0.21	0.34 ± 0.1	1.22 ± 0.3*

The lungs were isolated and perfused *in vitro* with Krebs solution at 10 ml min^{-1} and challenged with bolus doses of ovalbumin in 0.2 ml saline. Perfusate was collected for 10 min pre- and post-challenge and mediator concentrations are expressed as the increase following challenge (n = 5 or 6). * $P < 0.05$; † $P < 0.01$ compared to lung perfused and challenge via the pulmonary artery.

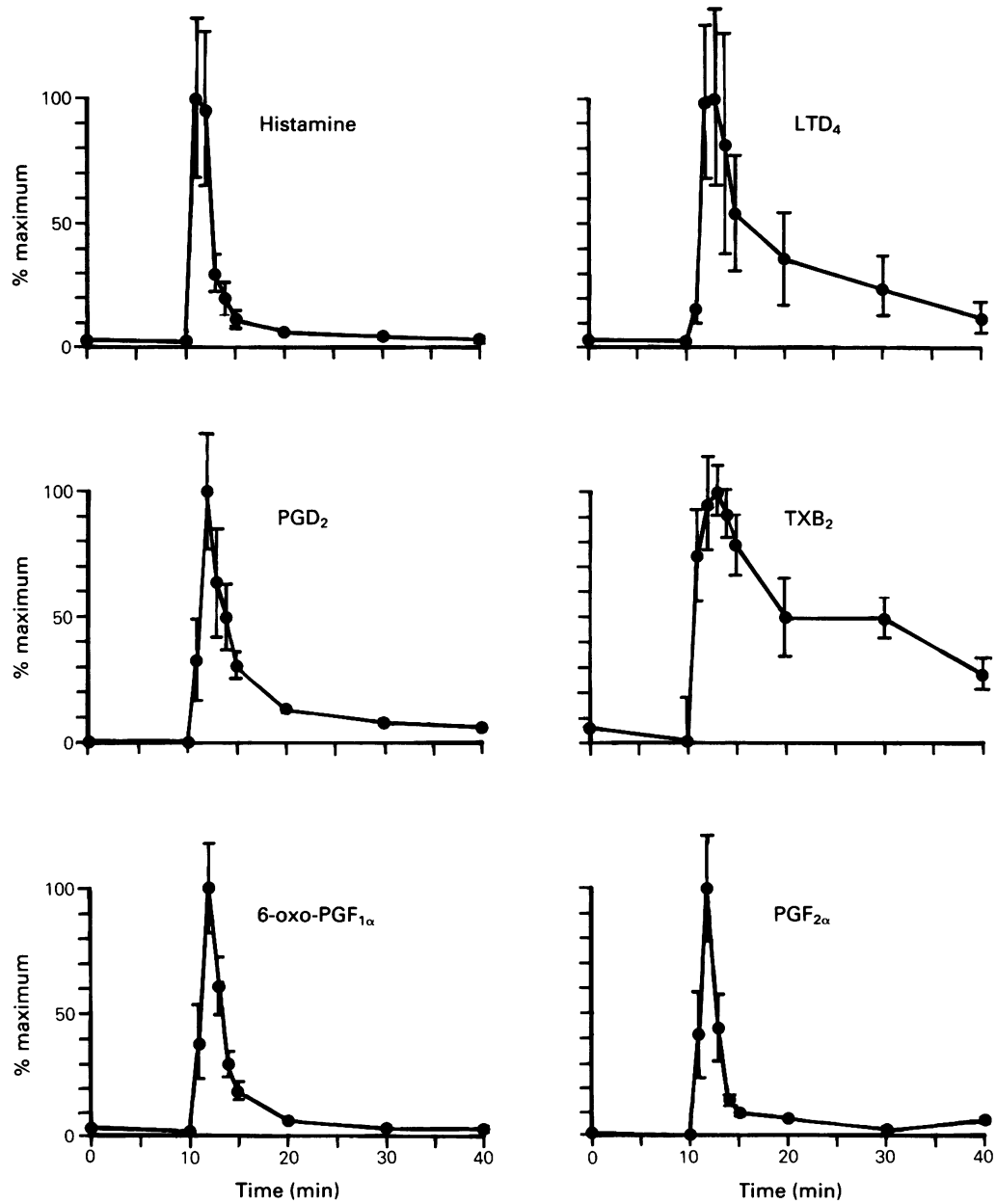

Figure 2 Time-course of histamine and eicosanoid release from sensitized guinea-pig isolated perfused lung challenged with 200 μg ovalbumin injected into the pulmonary circulation at 10 min. The results are expressed as a % of maximum rates of release and each point is the mean of 4–6 observations with s.e.mean shown by vertical lines. Peak rates of release (100%) were (ng min^{-1}) histamine 7450 ± 2450, leukotriene D_4 (LTD_4) 20.7 ± 7.6, prostaglandin D_2 (PGD_2) 259.5 ± 60.6, thromboxane B_2 (TXB_2) 474 ± 47.9, 6-oxo-$PGF_{1\alpha}$ 220 ± 39.9, $PGF_{2\alpha}$ 48.6 ± 9.8, PGE_2 51.7 ± 13.6. The time courses of prostaglandin release were almost indistinguishable from that of histamine.

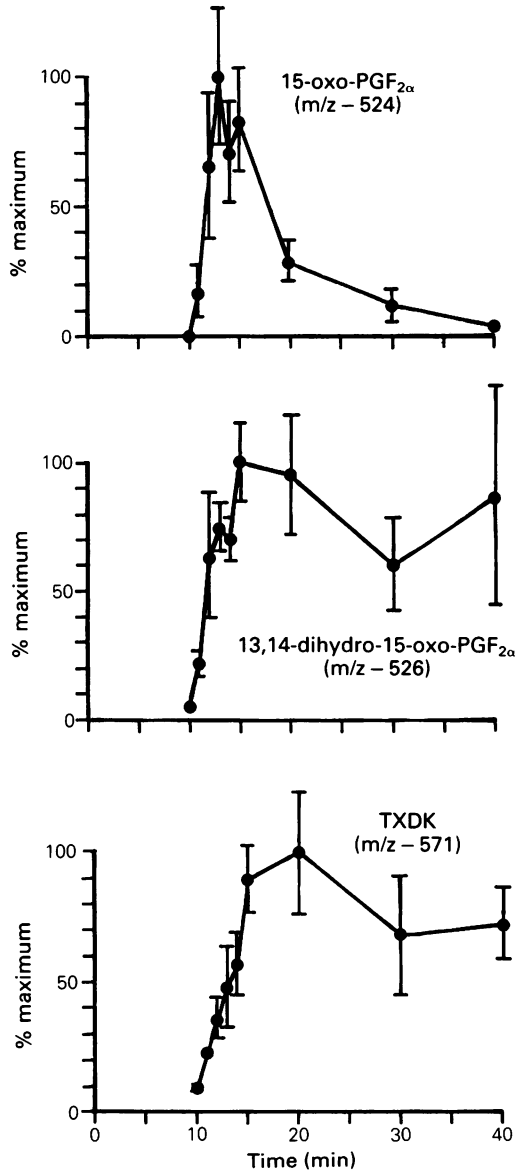

Figure 3 Time course of release of the major prostanoid metabolites from sensitized guinea-pig perfused lung following a 200 μg ovalbumin challenge at 10 min. Results are expressed as a % of maximum rates of release in 3 experiments with vertical lines showing s.e. mean.

LTD_4 and histamine concentrations by either route; $PGF_{2\alpha}$ and PGE_2 concentrations, however, were greater when the antigen was administered into the airway. With the exception of $PGF_{2\alpha}$ concentrations, eicosanoid production relative to histamine was similar regardless of the route of challenge.

When analysed by gc-nici/ms over the mass range 100 to 700, ions were detected having m/z values of -571, -526 and -524 with retention times identical to those of authentic 13,14-dihydro-15-oxo-TXB_2 (TXDK), 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ and 15-oxo-$PGF_{2\alpha}$ respectively. Due to the absence of suitable internal deuterated standards, however, co-chromatography of these ions with those of other prostanoid metabolites such as 6,15-dioxo-13,14-dihydro-$PGF_{1\alpha}$, and 9α 11β-13,14-dihydro-15-oxo-PGF_2 cannot be excluded. In addition smaller amounts of 13,14 dihydro-15-oxo-PGE_2 (m/z -481) and 6,15-dioxo-$PGF_{1\alpha}$ (m/z -569) were detected. Appearance of the major metabolites m/z -571 and -526 into the perfusion fluid reached peak rates 5–10 min after antigen injection and were maintained at 60–70% of this level for the duration of the perfusion. Release of the metabolite m/z -524 reached a peak at 3–5 min and thereafter declined to resting levels by 30 min (Figure 3). Based on the deuterated TXB_2 internal standard peak, the release of metabolites with an m/z of -571 was approximately 60% of that of TXB_2. Similarly, based on the deuterated $PGF_{2\alpha}$ standard, peak rates of release of 15-oxo-$PGF_{2\alpha}$ and 13,14-dihydro-15-oxo-$PGF_{2\alpha}$ were approximately 5 times and 4 times greater than that of $PGF_{2\alpha}$.

Discussion

The release of histamine and eicosanoids following antigen challenge was related both to the intensity of the stimulus and to the route of challenge. At peak rates of release, histamine was the predominant spasmogen present in the perfusate and is likely, therefore, to dominate the early phase of the anaphylactic bronchoconstriction. The more prolonged release of LTD_4 and TXB_2, however, suggests that the sustained phase might be maintained by leukotriene and thromboxane release. Thromboxane B_2 and PGD_2 were the principal cyclo-oxygenase products and their release, together with LTD_4, was related to histamine concentrations. The ratio of prostanoid concentrations relative to histamine (when expressed as a % of maximum release) however were greater at the lower antigen doses.

Histamine, PGD_2 and LTC_4 are the major products of mast cell activation (Peters *et al.*, 1985); however, whilst TXB_2 has been reported to be released from rat and human mast cells *in vitro* (Lewis *et al.*, 1982) its proportion relative to PGD_2 is small. We have found TXB_2 release to predominate and to be more sustained than that of the other eicosanoids, suggesting that it may be derived from non-mast cell

sources. Whilst there is a linear relationship between concentrations of mast cell products and TXB_2, the deviation of the intercept from the origin need not indicate that TXB_2 release is secondary to mast cell activation but may suggest the immunological activation of different cell types. The observation that at lower ovalbumin doses thromboxane B_2 release relative to histamine was greater than at the higher doses suggests that biologically active amounts of thromboxane may be released at low degrees of mast cell activation.

The profile of release of eicosanoids from guinea-pig lung has been suggested to reflect differences in sites of action of the stimulus and on the population of cells stimulated (Bakhle *et al.*, 1985a,b). We have demonstrated that at low doses of antigen the release of all mediators except LTD_4 and PGE_2, was greater when the lung was perfused and challenged via the trachea. There was, however, a proportionally greater increase in $PGF_{2\alpha}$ release. This increase in prostanoid release following challenge to the airway may be attributable to an increased accessibility of the antigen to the population of cells responsible for mediator release. The lung, however, is a major site of prostaglandin inactivation (Mathé *et al.*, 1977; Robinson & Hoult, 1982) and it is possible that the elevated prostaglandin levels (in contrast to unchanged LTD_4 concentrations) of airway versus arterial challenge reflects a reduced pulmonary catabolism of prostanoids released into the airway. Although it has been reported previously that prostaglandin inactivation is less efficient in the tracheobronchial tree (Mathé *et al.*, 1977), this does not account for the increased histamine concentrations following airway challenge. Furthermore, whilst $PGF_{2\alpha}$ concentrations remained greater following airway challenge with high doses of antigen, concentrations of the other mediators measured were similar regardless of the route of administration. The implication of these observations, therefore is that antigen delivered into the airway has a greater accessibility to those cells responsible for release of inflammatory mediators than when administered via the pulmonary circulation.

Finally, in addition to the primary prostanoids a number of prostaglandin metabolites were also detected in lung perfused via the vascular bed. Whilst the identity of the masses found with m/z values of -569 and -481 were confirmed as 6,15-dioxo-$PGF_{1\alpha}$, and 13,14 dihydro-15-oxo-PGE_2 by the co-chromatography of authentic standards, the identity of the masses in -571 and -526 are less certain due to the possible co-elution of 6,15-dioxo-13,14 dihydro-$PGF_{1\alpha}$ and 9α,11β-13,14 dihydro-15-oxo-PGF_2 with TXDK and the 13,14 dihydro-15-oxo-metabolite of $PGF_{2\alpha}$ respectively. The time course and amount of these metabolites released, however, indicates that measurement of the primary prostanoids alone underestimates their total formation. Furthermore the prolonged release of the metabolites in m/z -526 compared to the rather transient release of the possible parent compounds suggests either rapid uptake followed by metabolism (Robinson & Hoult, 1982) with a diffusion-limited release of metabolite or conversely, intracellular metabolism of the parent prostanoid prior to release. The latter possibility might serve as a regulatory mechanism limiting release of the biologically active prostanoid.

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