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TOTAL PROFILING BY GC/NICIMS OF THE MAJOR CYCLO-OXYGENASE PRODUCTS FROM ANTIGEN AND LEUKOTRIENE-CHALLENGED GUINEA-PIG LUNG

CLIVE ROBINSON*, J. R. S. HOULT†, KEITH A. WADDELL, IAN A. BLAIR and COLIN T. DOLLERY

Department of Clinical Pharmacology, Royal Postgraduate Medical School, Ducane Road, London W12 0HS, U.K.; † Department of Pharmacology, King's College, Strand, London WC2R 2LS, U.K.

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Abstract—Separation and quantitation of all the major cyclo-oxygenase products in perfused guinea-pig lungs challenged with antigen or leukotrienes C_4 and D_4 were achieved using a novel combined capillary column gas chromatography/negative ion chemical ionization mass spectrometric (GC/NICIMS) method. In descending order of magnitude, unchallenged lungs released thromboxane B_2 (TXB_2) plus its pulmonary metabolite (TXDK) > 6-keto-$PGF_{1\alpha}$ plus its 13,14-dihydro-15-keto metabolite ($K_2H_1F_{1\alpha}$) > PGE_2 plus $PGF_{2\alpha}$ > PGD_2; after ovalbumin anaphylaxis there were increases of ×26 in TXB_2 plus TXDK, ×28 in PGD_2 and histamine (measured fluorometrically) but of only ×3 in 6-keto-$PGF_{1\alpha}$ plus $K_2H_2F_{1\alpha}$ and PGE_2 plus $PGF_{2\alpha}$. FPL55712 treatment greatly reduced the release of TXB_2 and 6-keto-$PGF_{1\alpha}$ and their metabolites (showing this to be a secondary effect mediated by leukotriene action) but did not affect PGD_2 output. LTC_4 and LTD_4 themselves induced the release of TXB_2 and TXDK, as did bradykinin, but neither substance caused appreciable PGD_2 release. Aside from illustrating the great value of the GC/NICIMS method for simultaneously determining all cyclo-oxygenase products, the main conclusions are: (i) PGD_2 may be an *in vitro* marker for activation of lung inflammatory cells; (ii) prostacyclin and thromboxanes are actively metabolized *in situ* in the lung; and (iii) 'pathological subversion' of pulmonary function by anaphylaxis, leukotrienes or bradykinin principally causes thromboxane release from unknown target cells, with a smaller release of prostacyclin which may be compensatory in nature.

Interaction of specific antigen in the lung with cell-bound reaginic antibody leads to the release of preformed histamine from mast cells and the generation of an array of potent newly synthesized lipid mediators (for review, see ref. [1]). The large majority of the studies on the release of arachidonic acid metabolites from the lung has been performed using bioassay methods. Although of unquestionable value in detecting novel biological activity [2], such techniques have low analytical resolution and cannot detect inactive metabolites of active compounds. More recently, packed column gas chromatography/electron impact mass spectrometry (GC/EIMS) confirmed the presence of prostanoid mediators in shocked guinea-pig lung effluents [3], but this method did not employ internal standards for quantitative analysis and was insufficiently sensitive to detect arachidonate metabolites except in pooled perfusate from several hundred animals. Furthermore, in the latter study [3] there was no attempt to measure the release of prostaglandin D_2 which may be of great importance in type I hypersensitivity reactions [1].

As a knowledge of the detailed relationships between the synthesis and metabolism of released mediators is fundamental to an understanding of the expression of their biological activity, we now provide the first complete and truly quantitative profile of anaphylactically generated cyclo-oxygenase products using a novel combined capillary column gas chromatography/negative ion chemical ionization mass spectrometric (GC/NICIMS) technique [4, 5].

Our aim was to quantify the principal cyclo-oxygenase products released in ovalbumin-induced IgG-dependent guinea-pig pulmonary anaphylaxis: thromboxane A_2 (TXA_2) and its hydrolysis product TXB_2 or pulmonary metabolite 13,14-dihydro-15-keto-TXB_2 (TXDK [6]); prostacyclin as its hydrolysis product 6-keto-$PGF_{1\alpha}$ or metabolite 13,14-dihydro-6,15-diketo-$PGF_{1\alpha}$($K_2H_2F_{1\alpha}$); prostaglandins E_2 and $F_{2\alpha}$ and their 15-keto or 13,14-dihydro-15-keto pulmonary metabolites and prostaglandin D_2. We also tested the effects of bradykinin and exogenous leukotrienes LTC_4 and LTD_4 as triggers for pulmonary cyclo-oxygenase metabolism. Finally, we used the histamine H_1 receptor antagonist mepyramine and leukotriene antagonist FPL 55712 [7] as modifying agents to investigate the role of endogenous histamine and leukotrienes in anaphylactic prostanoid release. In some experiments histamine was also measured.

A preliminary account of this work has appeared elsewhere [8].

MATERIALS AND METHODS

Lung perfusion. Isolated, ventilated lungs from

* To whom correspondence should be addressed. Present address: Department of Clinical Pharmacology, Faculty of Medicine, Centre Block, Southampton General Hospital, Tremona Road, Southampton SO9 4XY, U.K.

male Dunkin–Hartley guinea-pigs (400–550 g) were perfused at 10 ml/min via the pulmonary artery with warmed, well-oxygenated Krebs' solution [6]. After a 20 min equilibration period the perfusate was collected for 9 min periods as described in Table 1. Some animals were sensitized 14–28 days beforehand with ovalbumin [6] or sham-sensitized with 0.9% saline alone. Lungs were challenged with intra-arterial bolus injections with the four agents as indicated in Table 1.

Extraction and analysis of prostanoids. After the addition of internal deuterated standards of PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ [4, 5], 4 ml aliquots of perfusate were adjusted to pH 3.0 and extracted into 7 ml ethyl acetate using C_{18}-reverse phase Sep-Pak cartridges that had been pre-conditioned with methanol (5 ml) and water (5 ml). The ethyl acetate fractions were then further chromatographed on silica straight-phase Sep-Pak cartridges [4, 5]. Samples were then derivatized: keto functions converted to methoximes, carboxyl groups to pentafluorobenzyl (PFB) esters and hydroxyl functions to trimethylsilyl ethers [4–6]. Analyses were performed on a Finnigan 4000 GC/MS system using a Chrompak Sil-5 fused silica capillary GC column and operated in the NICI mode with methane as reagent gas.

GC column temperature was programmed from 198° to 240° at 20°/min, then 240°–320° at 3°/min. Helium (1 ml/min) was used as GC carrier gas. Ion source pressure was 27 Pa. Ionizer temperature was 250° and electron energy was 100 eV. The analytical method used has been described in detail elsewhere [4, 5].

Quantitative determination of each prostanoid was carried out in the selected ion monitoring mode by comparison of the peak areas (peak height for 6,15-diketo-13,14-dihydro-$PGF_{1\alpha}$) of the appropriate $[M - C_7H_2F_5]^-$ ion for each prostanoid with that of the corresponding deuterated analogue. $[^2H_4]PGE_2$ was used as the internal standard for PGD_2 and $[^2H_4]$-6-keto-$PGF_{1\alpha}$ for TXB_2 and TXDK as deuterated standards for these compounds were not available [4, 5]. Assay sensitivity for the prostanoids was 1–8 pg on column with a signal to noise ratio of >2:1. Recovery through extraction and derivatization was generally greater than 70%, except for TXDK (58%).

Histamine assay. Histamine was analysed spectrophotofluorimetrically using the method of Shore *et al.* [9] on a Technicon autoanalyser.

Materials. Sources of the compounds used were ovalbumin (BDH, Poole, Dorset, U.K.), bradykinin triacetate and mepyramine maleate (Sigma London Ltd., Poole, Dorset, U.K.), FPL 55712 (Fisons PLC, Loughborough, U.K.), LTC_4/LTD_4 (Merck–Frosst Laboratories, Pointe Claire, Canada) and prostaglandins (Upjohn Co., Kalamazoo, MI). Sep-Paks were purchased from Waters Associates (Northwich, Cheshire, U.K.).

Statistics. Data are presented as mean values ± S.E.M., and the significance of differences evaluated by Student's unpaired *t*-test.

RESULTS

The complete results and protocols for the nine series of experiments are set out in Table 1. In all cases the data show the total amount of substance released per challenge (i.e. in 90 ml perfusate) and have been corrected for percentage recovery through extraction and derivatization.

Under resting conditions there was a continuous efflux of small amounts of all the prostanoids (lines A in Table 1) with thromboxane- and prostacyclin-derived products dominant. In 47 lungs the total thromboxane (TXB_2 plus TXDK) release in 9 min was 81.8 ± 4.3 ng per lung, of which 72.5 ± 2.4% was the TXDK metabolite. The corresponding figures for prostacyclin release were 77.7 ± 6.8 ng, measured as the sum of 6-keto-$PGF_{1\alpha}$ and $K_2H_2F_{1\alpha}$ (35.9 ± 2.4% as metabolite), confirming that the isolated lung generates large amounts of prostacyclin [10]. Rather smaller amounts of primary prostaglandins were also released: 6.3 ± 0.6 ng $PGE_2/PGF_{2\alpha}$ (cf. refs. [2, 11]) and 2.5 ± 0.2 ng PGD_2. We found no appreciable differences between basal prostanoid release in lungs taken from sham-sensitized or ovalbumin-sensitized animals (cf. line 2A with 1A), contrary to a previous report based on radioimmunoassay of the quantitatively minor E- and F-type PGs [11].

Anaphylaxis caused very large changes in the release of thromboxanes (26-fold increase, comparing 2B with 2A, $P < 0.001$, cf. refs. [2, 3]), PGD_2 (28-fold increase, $P < 0.01$) and histamine (10-fold increase from 110 ± 62 to 1080 ± 369 ng/90 ml, $P < 0.05$, cf. refs. [2, 12]), with lesser increases in prostacyclin metabolites (up by 3.4-fold, $P < 0.001$, cf. ref. [3]) and PGE_2 plus $PGF_{2\alpha}$ (2- to 8-fold, $P < 0.001$). Of the thromboxanes, 76.5 ± 2.6% was present as the metabolite TXDK, whereas $K_2H_2F_{1\alpha}$ represented 46.8 ± 8.9% of the total prostacyclin-derived material. As found earlier [2], repeated ovalbumin challenges continued to cause prostanoid release, although we found that the first has the greatest effect (experiment 3). Ovalbumin challenge of lungs from sham-sensitized animals had no effect (experiment 1).

We also obtained evidence for the release of small amounts of other compounds in addition to those described. For example, when samples were scanned over the 100–800 mass range, ions were observed at *m/z* values of −524, −481 and −526 with retention times corresponding to authentic 15-keto-$PGF_{2\alpha}$ (9.93 min) and the 13,14-dihydro-15-keto metabolites of PGE_2 (two isomers with retention times of 10.12 and 10.30 min) and $PGF_{2\alpha}$ (10.65 min) respectively, but in view of the very small amounts routine quantitation was not attempted. In addition, there was a weak ion at *m/z* − 569 (retention time 11.52 min) which in theory could be the 15-keto metabolite of TXB_2*, and also an ion at *m/z* −479 which had an identical retention time to leukotriene B_4.

PGD_2 is now recognized as the major cyclo-oxygenase metabolite released upon challenge of rat serosal and human lung mast cells [13, 14], but no pharmacological studies have been performed on its release from intact lungs. Thus we used FPL 55712

* This compound has never been detected in biological samples.

Table 1. Release of cyclo-oxygenase products (prostanoids) from isolated perfused guinea-pig lungs

Type and No. lungs	Collection periods A B C D	Experiment No.	PGD_2	$PGE_2 + PGF_{2\alpha}$ (ng/90 ml ± S.E.M.)	TXB_2 + TXDK	$6KF_{1\alpha} + K_2H_2F_{1\alpha}$	Total cyclo-oxygenase yield (ng)
Sham-sensitized (5)	1	1A	4.0 ± 1.7	3.9 ± 0.8	83.5 ± 15.4	54.1 ± 9.7	146
		1B	2.7 ± 0.8	1.8 ± 0.7	123.1 ± 19.6	42.4 ± 5.0	170
Sensitized (10)	2	2A	1.9 ± 0.7	4.5 ± 0.8	74.8 ± 15.9	75.4 ± 11.2	157
		2B	52.8 ± 16.1	12.7 ± 3.8	1917.0 ± 330.0	258.0 ± 45.9	2241
Sensitized (3)	3	3A	0.8 ± 0.4	4.9 ± 0.8	128.2 ± 18.0	96.7 ± 11.2	231
		3B	14.7 ± 5.5	7.3 ± 2.1	1774.0 ± 469.0	357.1 ± 105.9	2153
		3C	8.3 ± 2.1	4.8 ± 1.2	1589.0 ± 128.0	217.3 ± 44.3	1819
		3D	8.8 ± 4.7	6.7 ± 2.1	1471.0 ± 575.0	189.3 ± 30.6	1676
Sensitized (6)	4	4A	2.9 ± 1.9	16.7 ± 4.8	48.8 ± 7.8	39.4 ± 2.5	108
	FPL 55712, 10 μM	4B	58.8 ± 21.1	7.0 ± 2.4	207.5 ± 58.9	52.1 ± 16.2	325
		4C	25.6 ± 4.1	9.7 ± 3.9	981.1 ± 289.0	374.0 ± 96.1	1390
Sensitized (3)	5	5A	< 1.5	5.6 ± 2.8	105.7 ± 61.7	47.1 ± 6.3	160
	FPL 55712, 0.5 μM	5B	24.6 ± 4.1	10.5 ± 2.2	635.0 ± 137.0	54.9 ± 9.6	725
Sensitized (5)	6	6A	2.1 ± 1.5	7.0 ± 2.9	133.0 ± 41.9	195.2 ± 56.3	337
	Mepyramine 10 μM	6B	34.6 ± 12.2	35.7 ± 6.9	2515.0 ± 550.0	414.1 ± 119.6	2999
Sensitized (3)	7	7A	3.9 ± 12.2	5.8 ± 0.7	34.8 ± 14.0	62.1 ± 18.7	107
	Mepyramine, 10 μM + FPL 55712, 10 μM	7B	48.2 ± 22.9	7.9 ± 2.8	266.0 ± 128.0	66.9 ± 25.4	389
Sham-sensitized (6)	8	8A	4.0 ± 1.7	3.9 ± 0.8	83.5 ± 7.0	54.1 ± 9.7	146
		8B	5.7 ± 2.0	3.4 ± 1.7	713.8 ± 162.6	367.1 ± 78.5	1090
Unsensitized (6)	9	9A	1.4 ± 0.9	3.8 ± 0.9	69.2 ± 25.3	78.7 ± 12.0	153
		9B	4.9 ± 2.6	4.3 ± 0.9	190.5 ± 48.9	139.7 ± 19.2	339
		9C	4.2 ± 2.3	6.5 ± 0.7	162.0 ± 30.5	77.6 ± 10.6	251
	9 min (= 90 ml) Scale:	*m/z* value of ion	524	524 + 569	614 + 571	614 + 571	
		Retention time (min)	10.13	10.35 + 9.78	10.97 + 11.55	11.35 + 11.66	

The left side of the table shows the nine experimental protocols, indicating the various collection periods and drug treatments. Lungs of male Dunkin–Hartley guinea-pigs were perfused as described and the perfusate was collected for 9 min (= 90 ml). Some animals were sensitized 14–28 days beforehand with ovalbumin [6] or sham-sensitized with vehicle alone. Lungs were challenged with intra-arterial bolus injections with the four agents as indicated by the symbols below. Prostanoids were analysed by GC/NICIMS as outlined in Materials and Methods. The right side of the table gives the total amounts present in the 90 ml perfusate, together with the *m/z* values for the ions considered and their retention times.

Challenge symbols: ▲ 200 μg ovalbumin, △ 2 μg bradykinin, □ 1 ng (= 2 pmole) LTD_4, ■ 1.25 ng (= 2 pmole) LTC_4.

and mepyramine to investigate whether endogenous leukotrienes or histamine contribute to PGD_2 release. Co-perfusion of FPL 55712 at 10 μM (experiment 4) did not greatly alter basal output of prostanoids (4A) but significantly reduced anaphylactic thromboxane ($P < 0.002$, cf. 4B with 2B), prostacyclin ($P < 0.01$) and PGE_2 plus $F_{2\alpha}$ release ($P < 0.05$), whereas PGD_2 was not changed. A lower concentration of FPL 55712 (0.5 μM, experiment 5) also blunted the usual anaphylactic release of thromboxane- ($P < 0.05$) and prostacyclin-derived ($P < 0.05$) products. PGD_2 was again essentially unaffected by this manipulation.

Mepyramine at 10 μM (experiment 6) did not significantly reduce the release of any of the mediators studied; rather there were non-significant increases (cf. 6B with 2B, but $P < 0.001$ for PGE_2 plus $PGF_{2\alpha}$ although basal levels were higher than usual in these lungs, 6A). This suggests that mast cell-derived histamine does not activate significantly the metabolism of arachidonic acid during pulmonary anaphylaxis. Moreover, in combination (experiment 7) mepyramine did not modify the inhibitory effect of FPL 55712 (7B vs 4B).

In experiment 9 we tested the effects of low doses of synthetic LTD_4 and LTC_4 administered sequentially. In all lungs tested there was an increased output of thromboxane- ($P < 0.05$) and prostacyclin-derived products ($P < 0.02$ in 9B) although these effects were smaller than after anaphylactic challenge (cf. 9B, 9C with 2B).

Although widely employed as a specific leukotriene antagonist, FPL 55712 may possess other pharmacological properties. At high concentrations in broken cell preparations it inhibits thromboxane synthetase [15], although this was not the case in the experiments described here because at the highest concentration used (10 μM) challenge of anaphylactically shocked lungs with bradykinin (4C) still led to a considerable generation of TXB_2 plus TXDK ($P < 0.05$ compared to 4A, 4B) and 6-keto-$PGF_{1\alpha}$ ($P < 0.01$) as well as PGD_2. The latter release may be due to spill-over from the ovalbumin challenge (4B) since bradykinin does not release PGD_2 (8B) despite being an effective stimulus for the release of thromboxanes [16].

Two groups [15, 17] have shown that FPL 55712 also inhibits mast cell histamine release *in vitro* in certain systems. Our experiments extend this finding to the intact guinea-pig lung, as in the presence of 0.5 or 10 μM FPL 55712 the histamine release in experiments 4B and 5B was almost totally suppressed: 57 ± 17 and 60 ± 17 ng/90 ml, respectively, cf. 1080 ± 369 ng/90 ml in 2B, $P < 0.001$. The mechanism of this effect which leaves the formation of PGD_2 unaltered is not known however.

DISCUSSION

The release of arachidonic acid metabolites from guinea-pig lung has been widely studied. However, until now it has not been possible to quantify simultaneously all the important cyclo-oxygenase products released from lungs by various stimuli. We have employed GC/NICIMS to measure prostanoids released from single lungs during pulmonary anaphylaxis or following challenge with leukotrienes and bradykinin. The sensitivity and specificity of this newly developed technique are sufficiently great to allow the measurement of prostanoid release under basal conditions before the challenges, most notably allowing us to show that there is a continuous efflux of PGD_2, TXDK and $K_2H_2F_{1\alpha}$, none of which has previously been detected in control lung perfusates.

As expected, there were large increases in the output of cyclo-oxygenase products and histamine during anaphylaxis, but a striking feature was the 28-fold increase in the synthesis and release of PGD_2. Although this prostaglandin is the single most abundant cyclo-oxygenase product released from isolated activated mast cells [13, 14], it is not known whether these cells are its sole source in intact lung. Our experiments with mepyramine and FPL 55712 demonstrate that the observed release of PGD_2 in anaphylaxis is unlikely to be secondary to receptor-mediated actions of histamine and leukotrienes, respectively. Thus these data support our earlier suggestion [8] that PGD_2 release may be indicative of mast cell activation in intact lung and that PGD_2 could be used as a marker. However, the possibility that some as yet unidentified mast cell-derived factor—possibly analogous to prostaglandin-generating factor of anaphylaxis [18]—contributes to the release of PGD_2 cannot be formally excluded. It is also possible that some PGD_2 may derive from alveolar macrophages activated through IgE or IgG complexes [19]. However, when considered with the observed propensity of purified, isolated tissue mast cells to generate PGD_2 it would seem more likely that the majority of PGD_2 seen in our experiments was mast cell-derived.

Assuming the selectivity of FPL 55712 as a leukotriene antagonist, our results in experiment 4 are consistent with the concept that leukotrienes generated in guinea-pig IgG-dependent pulmonary anaphylaxis trigger the release of further metabolites of arachidonic acid (prostacyclin, thromboxanes, but not PGD_2, as described above), by activating phospholipase(s) in secondary cells. The generation of thromboxane A_2 by SRS-A or leukotrienes is well recognized and has been shown by superfusion cascade bioassays [2, 20]; the present experiments confirm and quantify this by measurement of TXB_2 and the 13,14-dihydro-15-keto-metabolite (TXDK). In fact, the metabolite accounted for 86% of the total (data not shown), suggesting that previously used bioassay methods must considerably underestimate the extent of thromboxane generation: even if the conversion to metabolites occurs in the manner $TXA_2 \rightarrow TXB_2 \rightarrow$ TXDK rather than $TXA_2 \rightarrow$ TXA_2 metabolite $\rightarrow$ TXB_2 metabolite, this process must take place in the lung and not in the effluent, therefore leaving bioassay tissues to respond only to remaining TXA_2 before its hydrolysis to TXB_2.

This reservation applies to all other studies which have measured thromboxane-synthesizing capacity solely in terms of thromboxanes A_2 or B_2. Thus, claims of major species differences in the ability to synthesize thromboxanes [21–23] must be viewed as provisional.

Our experiments also provide direct evidence (lines 9B and 9C) that low concentrations of 6-sul-

phidopeptide leukotrienes also cause the generation of prostacyclin, although this might be inferred from studies on the inhibition of the vasodepressor effects of leukotrienes by cyclo-oxygenase inhibitors in the intact guinea-pig [24, 25]. This interaction is important in relation to the cardiovascular actions of leukotrienes and their potential functions as inflammatory mediators, and it should be tested further in the isolated guinea-pig lung using a range of doses, as well as in other intact vascular preparations and isolated endothelial cells.

It was perhaps surprising that mepyramine treatment did not affect the anaphylactically triggered release of arachidonate metabolites because we and others [2, 16, 26] have shown previously that histamine causes the generation of thromboxane A_2-like material (rabbit aorta-contracting substance) and TXB_2. However, these studies showed that doses of 1–10 μg histamine were needed, whereas in the present experiments 1.08 ± 0.37 μg were released over the 9 min collection period. Thus the amounts of anaphylactically released histamine may be insufficient to cause phospholipase activation.

It is also interesting that the three chemical triggers which we have used to stimulate arachidonate metabolism exert by far their largest effects on the formation of thromboxanes. If the values shown in Table 1 are summated, then thromboxanes (i.e. TXB_2 plus TXDK) account for 86% of the anaphylactic cyclo-oxygenase products, or 84% if the results with inhibitors (lines 4B, 5B, 6B and 7B) are taken into account. Prostacyclin-derived products account for 13% and PGD_2 1.1% or 2.1%. Similarly, in the case of bradykinin and leukotriene challenge, thromboxanes account for 68 and 61%, respectively, of the total cyclo-oxygenase yield. We can thus suggest, as others have done previously, that in general terms 'pathological subversion' of pulmonary function by treatment with exogenous pharmacologically active substances (in this case activators of phospholipase A_2) results in the preferential generation of thromboxanes which in the intact animal would exacerbate any vasoconstriction and bronchoconstriction produced by the trigger substances themselves. It may also be true that the release of prostacyclin and its metabolites, perhaps from cells distinct from those which synthesize thromboxanes, could represent a partial homeostatic response in that their actions oppose those of thromboxanes.

A final feature of our results is that substantial proportions of the stable hydrolysis products of thromboxane A_2 and prostacyclin were recovered in the form of their 13,14-dihydro-15-keto metabolites (TXDK and $K_2H_2F_{1\alpha}$, respectively). Metabolism of the thromboxanes was greater, and ranged from 72.5 to 86.0%. We have previously demonstrated that the isolated guinea-pig lung converts *ca* 30% exogenously administered TXB_2 to the TXDK metabolite [6, 27], but we cannot say whether conversion is by the same route during anaphylaxis or whether TXA_2 itself is enzymatically transformed. However, the clear implication from these and the previous experiments [6, 27] is that endogenous thromboxanes can be enzymatically converted to inactive products, presumably so as to restrict their actions to sites close to their release. This contrasts with the alternative view, namely that the actions of thromboxane A_2 are limited principally by non-enzymatic decomposition. On the face of it, this seems unlikely because on a biological time scale the half-life of *ca.* 30 sec is long, and certainly sufficient to allow systemic transfer to distant sites. Interestingly, the metabolite TXDK has recently been detected in human lung [28].

In conclusion, this GC/NICIMS assay provides complete data on the total spectrum of cyclo-oxygenase products released from challenged guinea-pig lung. The method promises to clarify our understanding of multicellular systems like the lung where prostanoid biosynthesis follows complex divergent cell-dependent pathways. They lead to multiple products and metabolites which cannot satisfactorily be resolved and quantified by previously used methods of bioassay and radio-immunoassay but are amenable to analysis by GC/NICIMS. The method also offers much scope for the investigation of the pharmacological effects of anti-allergic drugs on the pulmonary prostaglandin system.

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REFERENCES

1. R. A. Lewis and K. F. Austen, *Nature, Lond.* **293**, 103 (1981).
2. P. J. Piper and J. R. Vane, *Nature, Lond.* **223**, 29 (1969).
3. J. R. Boot, A. F. Cockerill, W. Dawson, D. N. B. Mallen and D. J. Osborne, *Int. Archs Allergy appl. Immun.* **57**, 159 (1978).
4. K. A. Waddell, S. E. Barrow, C. Robinson, M. A. Orchard, C. T. Dollery and I. A. Blair, *Biomed. Mass Spectrom.* (in press).
5. K. A. Waddell, C. Robinson, M. A. Orchard, S. E. Barrow, C. T. Dollery and I. A. Blair, *Int. J. Mass. Spectrom. Ion Phys.* **48**, 233 (1983).
6. C. Robinson, S. H. Peers, K. A. Waddell, I. A. Blair and J. R. S. Hoult, *Biochim. biophys. Acta* **712**, 315 (1982).
7. J. Augstein, J. B. Farmer, T. B. Lee, P. Sheard and M. L. Tattersall, *Nature, New Biol.* **245**, 215 (1973).
8. I. A. Blair, C. T. Dollery, M. Ennis, J. R. S. Hoult, C. Robinson and K. A. Waddell, *Br. J. Pharmac.* **78**, 49P (1983).
9. P. A. Shore, A. Burkhalter and V. H. Cohn, *J. Pharmac. exp. Ther.* **127**, 182 (1959).
10. R. J. Gryglewski, J. Korbut and A. Ocetkiewicz, *Nature, Lond.* **273**, 765 (1978).
11. A. A. Mathé, S.-S. Yen, R. Sohn and P. Hedqvist, *Biochem. Pharmac.* **26**, 181 (1977).
12. W. E. Brocklehurst, *J. Physiol., Lond.* **151**, 416 (1960).
13. L. J. Roberts II, R. A. Lewis, K. F. Austen and J. A. Oates, *Biochim. biophys. Acta* **575**, 185 (1979).
14. R. A. Lewis, N. A. Soter, P. T. Diamond, K. F. Austen, J. A. Oates and L. J. Roberts II, *J. Immun.* **129**, 1627 (1982).
15. A. F. Welton, W. C. Hope, L. D. Tobias and J. G. Hamilton, *Biochem. Pharmac.* **30**, 1378 (1981).
16. C. Robinson and J. R. S. Hoult, *Eur. J. Pharmac.* **64**, 333 (1980).

17. D. R. Buckle, D. J. Outred, J. W. Ross, H. Smith, R. J. Smith, B. A. Spicer and B. C. Gasson, *J. med. Chem.* **22**, 158 (1979).
18. L. Steel and M. Kaliner, *J. biol. Chem.* **256**, 12692 (1981).
19. W. Hsueh, C. S. Kuhn and P. Needleman, *Biochem. J.* **184**, 345 (1979).
20. P. J. Piper and M. N. Samhoun, *Br. J. Pharmac.* **77**, 267 (1982).
21. F. F. Sun, J. P. Chapman and J. C. McGuire, *Prostaglandins* **14**, 1055 (1977).
22. F. Al-Ubaidi and Y. S. Bakhle, *Eur. J. Pharmac.* **62**, 89 (1980).
23. T. E. Eling and A. I. Ally, *Clin. resp. Physiol.* **17**, 611 (1981).
24. A. Ueno, K. Tanaka and M. Katori, *Prostaglandins* **23**, 865 (1982).
25. P. Schiantarelli, S. Bongrani and G. Folco, *Eur. J. Pharmac.* **73**, 363 (1981).
26. F. Berti, G. C. Folco, S. Nicosia, C. Omini and R. Pasargiklian, *Br. J. Pharmac.* **65**, 629 (1979).
27. J. R. S. Hoult and C. Robinson, *Br. J. Pharmac.* **78**, 85 (1983).
28. S.-E. Dahlén, G. Hansson, P. Hedqvist, T. Björck, E. Granström and B. Dahlen, *Proc. natn. Acad. Sci. U.S.A.* **80**, 1712 (1983).