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The platelet-independent release of thromboxane A_2 by Paf-acether from guinea-pig lungs involves mechanisms distinct from those for leukotriene

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1 Intra-arterial injections of platelet-activating factor (Paf-acether, 10–300 ng) to the perfused guinea-pig lung induced a dose-related bronchoconstriction, followed by contraction of the rat aorta superfused with the lung effluent, indicating the release of thromboxane A_2 (TXA_2) activity. These effects were matched with injections of bradykinin (Bk) at 100–1000 ng, leukotriene C_4 (LTC_4) at 10–300 ng or arachidonic acid (AA) at 30–300 μg.

2 Repeated doses of Paf-acether led to a specific desensitization of the release of TXA_2, under conditions where Bk, LTC_4 and arachidonic acid retained their ability to release TXA_2.

3 Bronchoconstriction and the release of TXA_2 induced by Paf-acether were suppressed when the lungs were perfused with acetylsalicylic acid, but not with salicylic acid.

4 The phospholipase A_2 inhibitor, *p*-bromophenacyl bromide suppressed the release of TXA_2 by Bk, but did not interfere with its formation from AA, nor with its release with Paf-acether and LTC_4. The lipoxygenase inhibitor, nordihydroguaiaretic acid, inhibited to a similar extent the release of TXA_2 by Bk, LTC_4 and Paf-acether but also reduced directly the formation of TXA_2 from arachidonic acid, invalidating its use as a specific antilipoxygenase agent.

5 The leukotriene C_4/D_4 antagonist, FPL 55712, suppressed the TXA_2 releasing effects of LTC_4, and was completely inactive against Paf-acether, Bk or arachidonic acid.

6 The aerosol of Paf-acether was tested in the anaesthetized guinea-pig and resulted in bronchoconstriction, unaccompanied by thrombocytopenia. Unlike bronchoconstriction induced by intravenous Paf-acether, which is refractory to cyclo-oxygenase inhibitors, the effects of the aerosol were suppressed by aspirin. Platelet depletion, which blocks the intravenous effects of Paf-acether, failed to interfere with those of the aerosol.

7 Paf-acether induced a marked contraction of the superfused guinea-pig isolated parenchyma lung strip, which was followed by total and irreversible desensitization to itself. The contractile effect was not inhibited by aspirin or indomethacin, atropine, mepyramine, methysergide, phenoxybenzamine or propranolol, indicating that cyclo-oxygenase products, cholinergic stimuli, histamine, 5-hydroxytryptamine and catecholamine mechanisms are not involved.

8 Our results indicate that Paf-acether interacts with pulmonary sites distinct from those for Bk, LTC_4 or AA, since no cross-desensitization between Paf-acether and the other agonists was noted, *p*-bromophenacyl bromide inhibited Bk only and FPL 55712 inhibited only LTC_4. The phospholipase A_2 involved with the release of the arachidonate needed for the formation of TXA_2 by Paf-acether or LTC_4-stimulated lungs may differ from the enzyme accounting for its formation by Bk. The cellular sites with which Paf-acether interacts may also be distinct and less readily accessible to *p*-bromophenacyl bromide.

Introduction

Platelet-activating factor (Paf-acether, 1-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) injected intravenously into the anaesthetized guinea-pig induces a platelet-dependent bronchoconstriction (BC). Although this BC is not inhibited by a cyclo-oxygenase inhibitor, such as aspirin, or by the simul-

taneous addition of the 5-hydroxytryptamine (5-HT) antagonist methysergide with mepyramine, a histamine antagonist, the combination of aspirin, methysergide and mepyramine will abolish BC induced by Paf-acether (Vargaftig *et al.*, 1980; 1982), suggesting an indirect involvement of thromboxane A_2 (TXA_2). This led us to study whether Paf-acether releases TXA_2 from guinea-pig lungs, and since it did so, to compare its effectiveness with that of bradykinin (Bk) and leukotriene (LT) C_4, two recognised mediators of BC and TXA_2 release, (Collier & Shorley, 1960; Piper & Vane, 1969; Schiantarelli *et al.*, 1981; Sirois *et al.*, 1982), Bk being known to act through the activation of lung phospholipase A_2 (Vargaftig & DaoHai, 1972; Damas & Bourdon, 1975; Blackwell *et al.*, 1978).

Leukotrienes and Paf-acether may be involved in bronchial asthma, since they induce BC and can be released after immune stimuli from various relevant tissues and cells (Piper, 1983; Borgeat *et al.*, 1983; Benveniste & Vargaftig, 1983; Roubin *et al.*, 1983; Rankin *et al.*, 1983). Although intravenous administration of antigens is used for the testing of potential mediators and antagonists of BC, asthma is usually triggered by the inhalation of antigen and so studies were performed using aerosolised Paf-acether *in vivo*. Since this route gave markedly different results from those obtained when Paf-acether was given intravenously, additional studies on isolated superfused lung strips were carried out. Here, the agonists reach their sites of action by diffusion and so interact primarily with cells distinct from those reached by intravascular routes. Finally, potential inhibitors of the effects of Paf-acether were tested, in particular agents which inhibit phospholipase A_2.

Our results show that the activity of Paf-acether is dependent on the route of administration and involves the interaction with specific sites in the lung. TXA_2 plays a major role in inducing BC in platelet-free perfused lungs, or after aerosol *in vivo* but a comparatively minor role when BC is platelet-dependent, i.e. after intravenous injections of Paf-acether.

Method

Lung perfusion experiments

Hartley guinea-pigs of either sex (300–500 g) were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.p.); the carotid artery, the jugular vein and the trachea were cannulated and ventilation was started (Palmer miniature respiratory pump, 60 strokes min^{-1}) spontaneous breathing being arrested with pancuronium (4 mg kg^{-1}, i.v.). Following mid-thoracotomy the pulmonary artery was exposed, cannulated and perfused for 10 min with 50 ml of Krebs solution (30°C) containing 2.5 g l^{-1} of bovine serum albumin (Krebs-BSA), 10 μg of prostaglandin E_1 (PGE,) and 500 units of heparin. This procedure minimized spontaneous BC during the dissection, and was expected to prevent the formation of platelet aggregates in the microvessels. Lungs were then removed and suspended in a plastic chamber; perfusion with Krebs-BSA was immediately started at 10 ml min^{-1} (37°C). The trachea was connected to the respiratory pump, and a T piece inserted to record BC. The lung effluent superfused a rat aortic strip, which detects TXA_2 activity, as reported for rabbit arterial vessels (Piper & Vane, 1969; Vargaftig & Dao, 1971; Bunting *et al.*, 1976). In most experiments the rat aorta was perfused laterally with Krebs solution containing a mixture of antagonists to histamine (mepyramine, 10^{-6} M) acetylcholine (atropine, 1.5×10^{-6} M), catecholamines (phenoxybenzamine, 3×10^{-7} M and propranolol, 7×10^{-6} M), and to the *in situ* formation of prostaglandins (indomethacin, 3×10^{-6} M). The sensitivity of the rat aorta was tested periodically with a 2 min incubate of guinea-pig platelet rich plasma (PRP) and arachidonic acid (AA, 0.1–0.3 mM) as a source of TXA_2. The sensitivity of the recorder was adjusted for a full scale pen displacement for the aorta response to the TXA_2 source. Paired experiments were performed daily, one control and one treated lung, to ensure that failure to release TXA_2 from treated lungs did not result from treatment-independent factors.

Studies with isolated lung strips

Peripheral sub-pleural lung strips (2–2.5 cm long; 2–4 mm width) were dissected and mounted under 2 g tension. Usually 2–4 strips were superfused in parallel, with 10 ml min^{-1} of Krebs solution, containing the antagonists indicated above. The contractile activity was recorded isometrically.

In vivo *studies with aerosolized Paf-acether*

Pentobarbitone-anaesthetized guinea-pigs were prepared for the recording of bronchial resistance to inflation (here termed bronchoconstriction, BC) as described (Lefort & Vargaftig, 1978). Bronchial sensitivity was checked with 5-hydroxytryptamine (1–2 μg kg^{-1}, i.v.). After constant responses were obtained. the aerosol of Paf-acether was started and continued for 2 min. This was performed with a medical aeorosolator apparatus, with a concentration of 115–330 μg ml^{-1} of Paf-acether in the reservoir, which induced a BC equivalent to that due to 5-HT. The aerosol was induced by directing the output of the aerosol into the output tube of the respiratory pump.

Platelet depletion

Platelet depletion was induced by the injection of 0.5 ml kg^{-1} of an anti-platelet serum raised in rabbits (Lefort & Vargaftig, 1978). This resulted in the reduction of the number of circulating platelets by 90–95% within 1 h. Platelet counts were performed with a Coulter Counter ZBI, when required.

Materials

The following drugs were used: pentobarbitione (Lathévet), pancuronium (Pavulon, Organon), bovine serum albumin (BSA, Fraction V), 5-hydroxytryptamine, bradykinin, salicylic acid, arachidonic acid, indomethacin, nordihydroguaiaretic acid and phenoxybenzamine (Sigma), collagen (Hormon Chimie, Münich), aspirin (Aspégic, Egic), mepyramine and mepacrine (Rhone-Poulenc), atropine (Laboratoires Bruneau), methysergide (Sandoz), propranolol (ICI), FPL 55712 (sodium 7-[3(4-acetyl-3-hydroxy-2-probylphenoxy)-2-hydroxypropoxy]-4 oxo-8-propyl-4H-1-benzopyran-2-carboxylate a gift from Fisons), *p*-bromophenacyl bromide (Fluka), leukotriene C_4 and prostacyclin (a gift of Chinoin, Budapest). Paf-acether, in its natural *R* configuration, which is active on platelets, as well as its unnatural *S* configuration, which is inactive, were kindly provided by Professor J.J. Godfroid (Université de Paris VII).

Statistical analysis

All results are expressed as means ± s.e. mean and were analyzed by Student's test, $P < 0.05$ being considered as significant.

Results

Bronchoconstriction and release of thromboxane A_2 from perfused lungs

Bronchoconstriction resulted within 20–60 s of the injection of 10–300 ng Paf-acether into the pulmonary artery (Figure 1). This effect was not easily reversed, but in 3 instances the injection of 50 μg of isoprenaline 10 min after that of Paf-acether, led to the partial reversal of BC (not shown). This irreversibility invariably prevented the study of dose-dependency in the same lung preparation (Figure 1); however, when separate lungs were used for single injections of Paf-acether, the dose-dependency could be clearly seen (Table 1).

Bronchoconstriction induced by Paf-acether was followed by contraction of the perfused rat aorta, indicating that TXA_2 had been released from the lung (Figures 1 and 2). When 10, 30, 100 and 300 ng were injected into the same lung at 20 min intervals, a dose-dependent release of TXA_2 was observed, whereas higher concentrations (> 1 μg) were less

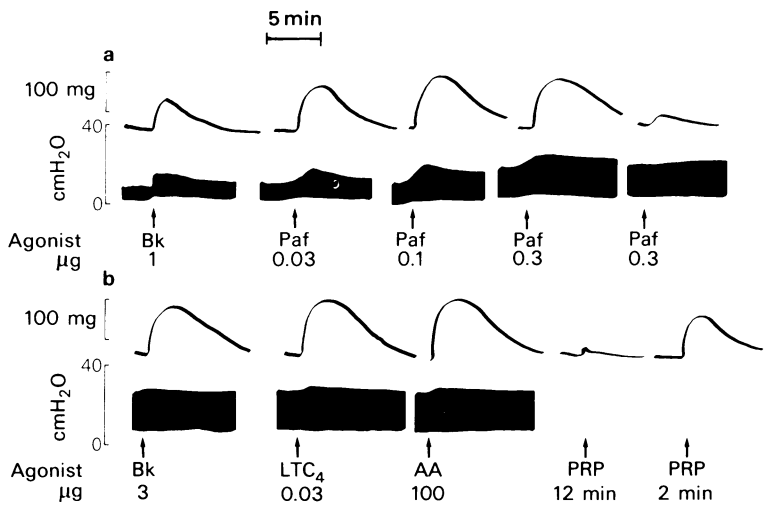

Figure 1 Release of thromboxane A_2 (TXA_2) activity following the intrapulmonary injections of bradykinin (Bk), of Paf-acether (Paf), of leukotriene C_4 (LTC_4) and of arachidonic acid (AA). In each panel the upper tracings show the contractions of rat aorta (scale = 100 mg) and the lower tracings show the bronchoconstrictor effect (scale = 40 cmH_2O). (a) Repeated injections of Paf-acether leading to desensitization of its TXA_2-releasing effect. (b) The releasing effects of bradykinin, of leukotriene C_4 and of arachidonic acid are maintained. PRP 12 min and 2 min indicate the direct injections onto the rat aorta of guinea-pig PRP incubated for 12 and 2 min, respectively, with 0.1 mM of AA. Thromboxane A_2 is detectable for 2 min, and fully degraded within 12 min. The doses of the various agonists are indicated in μg.

Table 1 Effects of intrapulmonary injections of Paf-acether

	Agonist	ng	Bronchoconstriction (%)	Thromboxane A_2 (%)
A	Paf-acether	10	54.7± 7.9 (7)	12.2± 4.1 (5)
	Paf-acether	30	81.2± 5.1 (5)	46.2±10.0 (5)
	Paf-acether	100	100 (5)	120.6±13.4 (5)
	Lyso-Paf-acether	1000	0 (3)	0 (3)
	S enantiomer	100	0 (3)	0 (3)
B	Paf-acether	10	41.8± 4.3 (7) NS	8.1± 2.1 (7) NS
	Paf-acether	30	81.8±11.3 (6) NS	49.2± 6.6 (6) NS
	Paf-acether	100	100 (6)	111.2±13 (6) NS

Values are mean ± s.e.mean. The amounts of Paf-acether, of its deacetylated product lyso-Paf-acether and of the unnatural enantiomer S, were injected into the pulmonary artery. Bronchoconstriction is expressed as a % of the maximal effect, and thromboxane A_2 as a % of the release induced by 1 μg of bradykinin. Control lungs indicated in (**A**) and lungs from platelet-depleted guinea-pigs in (**B**).
All animals had a drop of at least 90% of their arterial blood platelet content, when the values before and after the administration of anti-platelet serum were compared. Number of experiments in parentheses. NS = non significant difference when paired results of (**A**) and (**B**) were compared for the same doses of Paf-acether.

effective (Figures 1 and 2). Indeed, this led to desensitization of the TXA_2-releasing action of Paf-acether (Figure 1). Nevertheless, when a single dose of 1 μg of Paf-acether was injected into a perfused lung as a first stimulus, an aortic contraction of 870 ± 45 mg ($n = 4$) was obtained, showing that Paf-acether is as effective in releasing TXA_2 as is Bk, LTC_4 or AA (Figure 2). These agonists triggered the release of TXA_2 at concentrations of 300–3000 ng, 10–100 ng and 20–100 μg respectively (Figure 2). Figure 1 also shows that after desensitization to Paf-acether had been obtained, the other agonists

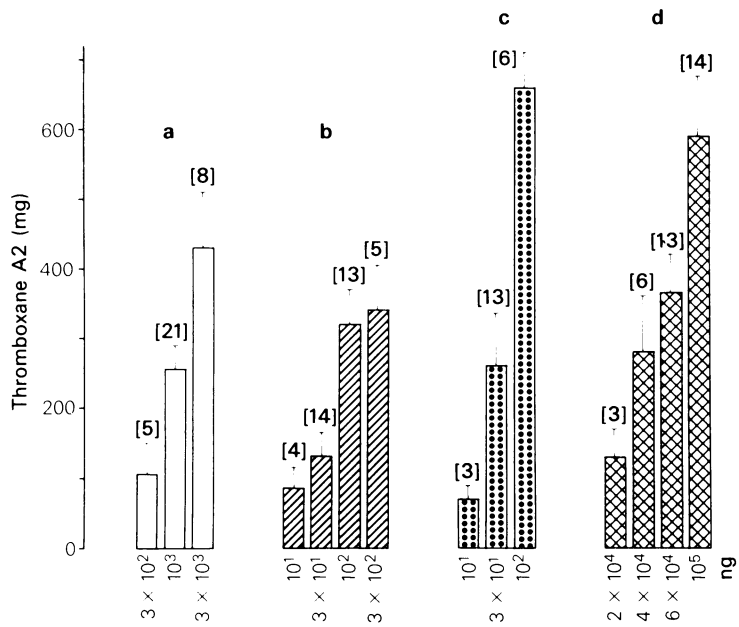

Figure 2 Dose-dependent release of thromboxane A_2 (TXA_2) activity induced by the intra-pulonary injections of bradykinin (a, open columns), Paf-acether (b, hatched columns), leukotriene C_4 (c, stippled columns) and arachidonic acid (d, crossed columns). The height of each column corresponds to the contractions of the rat aorta, in mg, upon stimulation with TXA_2 activity. Figures in parentheses indicate the number of experiments, and vertical lines indicate s.e.mean. Doses (ng) of the agonists are indicated below each column.

Table 2 Drug interference with the effects of intrapulmonary Paf-acether

Potential antagonists and dosage (M)	*ng*	*Bronchoconstriction* (%)	*Thromboxane* A_2 (%)
Salicylic acid	10	28.0 ± 8.7 (6)	11.2 ± 3.3 (6)
10^{-4}	30	66.2 ± 9.3 (6)	37.8 ± 6.0 (6)
	100	100 (3)	94.2 ± 9.1 (6)
Mepyramine	10	30.6 ± 3.2 (3)	5.5 ± 2.8 (3)
10^{-6}	30	63.3 ± 10.5 (3)	34.7 ± 6.6 (3)
	100	100 (3)	121.0 ± 13.4 (3)
Aspirin	10	7.0 ± 4.5* (4)	0* (4)
10^{-4}	30	9.0 ± 7.5* (4)	0* (4)
	100	4.25 ± 4.25* (4)	0* (4)
Mepacrine	10	0* (3)	0* (3)
5×10^{-5}	30	6.25 ± 6.25* (4)	0* (4)
	100	0* (4)	0* (4)

Values are mean ± s.e.mean. The amounts indicated of Paf-acether were injected into the pulmonary artery. Bronchoconstriction and the release of TxA_2 are expressed as in Table 1. The appropriate potential antagonists were added to the Krebs solution perfusing the lungs at the concentrations given, 30 min before injecting Paf-acether. Number of experiments in parentheses. *$P < 0.05$.

could still release TXA_2, suggesting that Paf-acether interacts with specific sites, unrelated to those for Bk and LTC_4. Finally, neither lyso-Paf-acether nor the inactive enantiomer of Paf-acether was effective in inducing BC or in releasing TXA_2.

Drug interference with the effects of Paf-acether

Bronchoconstriction and release of TXA_2 induced by Paf-acether were suppressed when the lungs were perfused with acetylsalicylic acid (ASA, 0.1 mM, 30 min), but not by salicylic acid at a similar concentration (Table 2). Reperfusion of the lungs with ASA-free Krebs, after ASA had been applied for 30 min and the block of the TXA_2-releasing effects of Paf-acether had been obtained, led to removal of the inhibition. It is of interest that the inhibitory effect of ASA is not removed when platelets are washed (Rosenberg *et al.*, 1971), whereas cyclo-oxygenase inhibition of the lung is reversible (Vargaftig & Dao, 1971). This finding, as well as the ineffectiveness of platelet-depletion in blocking the effects of Paf-acether (see below), confirmed that contaminant platelets are not the target cells for Paf-acether in the lungs. The histamine antagonist, mepyramine, in concentrations that suppress the TXA_2-releasing effects of histamine, failed to inhibit the effects of Paf-acether (Table 2).

Since the release of TXA_2 is said to depend upon the prior activation of phospholipase A_2 (Vargaftig & Dao Hai, 1972; Blackwell *et al.*, 1978), two phospholipase inhibitors were tested. As can be seen in Table 2, mepacrine (0.05 mM) suppressed BC and TXA_2 release induced by Paf-acether, Bk and LTC_4. However, *p*-bromophenacyl bromide when present at 0.4 mM in the Krebs solution perfusing the isolated lungs induced an immediate BC, followed by a marked lung oedema. In an attempt to minimize these apparently toxic effects, *p*-bromophenacyl bromide was added at 0.4 mM to the 50 ml of Krebs solution perfused through the pulmonary artery before the lung was removed from the animal. The presence of PGE_1 in the washing solution (see Methods) appeared to reduce these toxic effects, even though BC persisted. No further *p*-bromophenacyl bromide was infused after suspending the lungs in the chamber, which had the additional advantage of preventing it from being carried over onto the superfused rat aorta. The formation of TXA_2 from AA, as well as its release induced by LTC_4 or Paf-acether were unaffected by prior lung treatment with *p*-bromophenacyl bromide. In contrast, the release of TXA_2 by Bk was suppressed (Figure 3) thus clearly dissociating Bk from the other agonists. The leukotriene antagonist, FPL 55712, perfused directly into the isolated lungs (final concentration 0.01 mM; 10 min) inhibited the effects of LTC_4 but not those of AA, Bk or Paf-acether (Figure 4 and 5).

Finally, the lipoxygenase inhibitor nordihydroguaiaretic acid (0.01 mM) significantly reduced the release of TXA_2 by Bk, LTC_4 and Paf-acether (Figure 6) but at 0.03 mM was also found to inhibit TXA_2 formed directly from AA (Figure 6).

Role of platelets for the release of thromboxane A_2 from the isolated lungs

Despite the precautions taken during the lung dissection (PGE_1, heparin), the possibility remained that

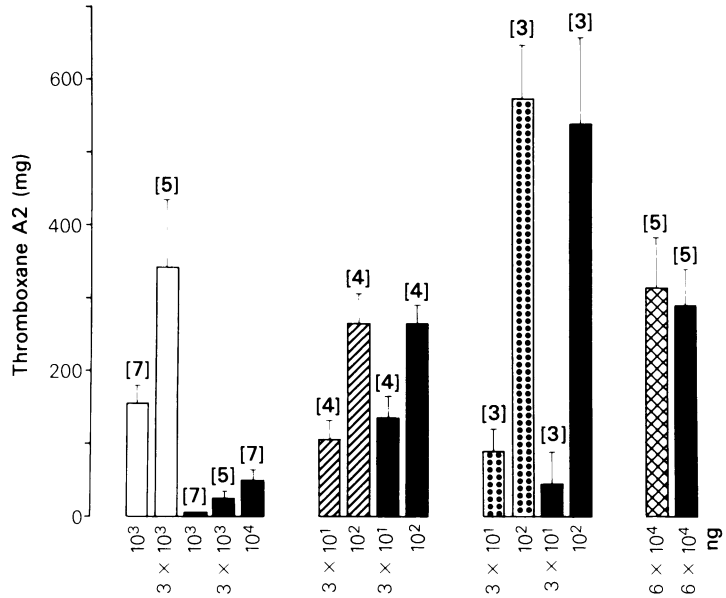

Figure 3 Inhibition by *p*-bromophenacyl bromide of bradykinin-induced release of thromboxane A_2 (TXA_2) activity from perfused lungs. The height of each column corresponds to the contractions of the rat aorta, in mg, upon stimulation with TXA_2 activity. The solid columns indicate the amounts obtained when the agonist was injected to *p*-bromophenacyl bromide-treated lungs. Other symbols as in Figure 2.

free platelets or micro-thormbi stuck to the endothelium might account for the formation of TXA_2 in response to Paf-acether. However, the platelet-stimulating agent, collagen, injected into the lungs at 100 μg failed to induce BC or to release TXA_2. Moreover, the effects of Paf-acether were unchanged both with respect to BC and to the release of TXA_2 when the lungs were collected from platelet-depleted animals (Table 1).

Experiments with aerosolized Paf-acether

The aerosol of Paf-acether was followed within 2–3 min by BC, which peaked at 5–8 min, persisted

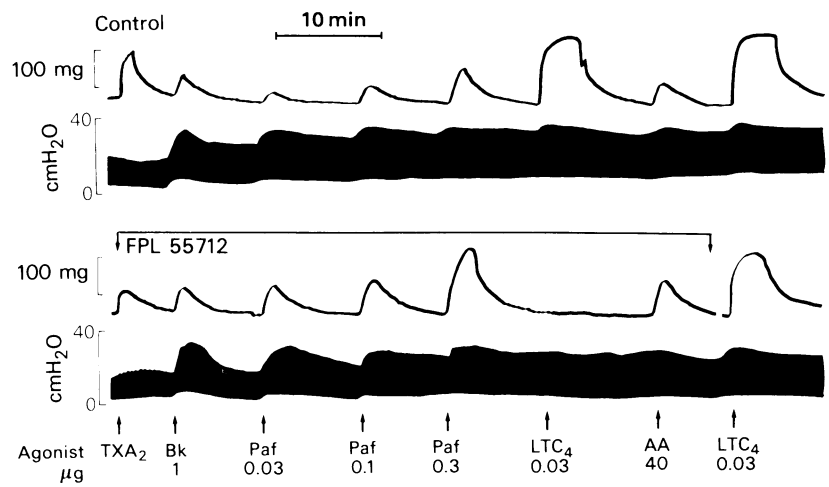

Figure 4 Effect of FPL 55712 on the release of thromboxane A_2 (TXA_2) activity by the perfused lungs. The upper panel is from the control lung preparation, and the lower panel from a paired lung perfused with FPL 55712 as indicated. Abbreviations, legends and scales as in Figure 1.

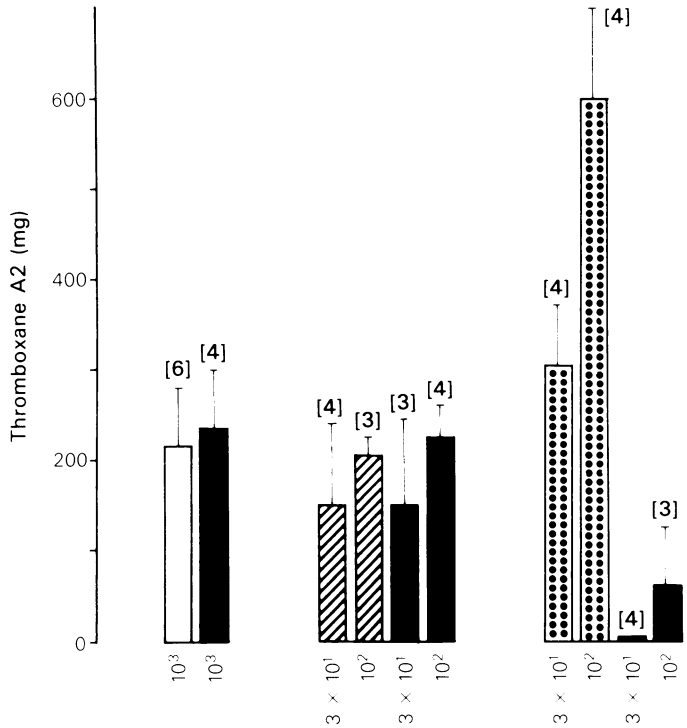

Figure 5 Inhibition by FPL 55712 of leukotriene C_4-induced release of thromboxane A_2 (TXA_2) activity from perfused lungs. Symbols as in Figure 2.

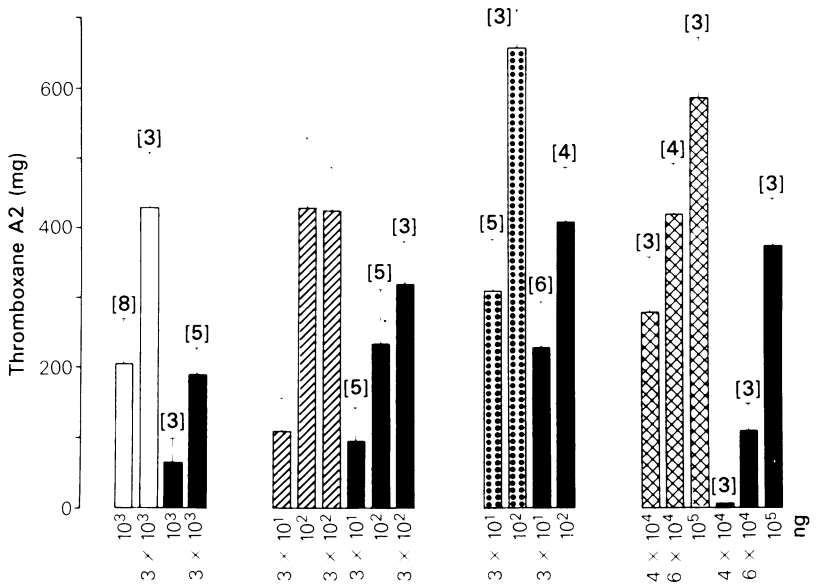

Figure 6 Inhibition by nordihydroguaiaretic acid of the release of thromboxane A_2 (TXA_2) from perfused lungs. Columns and symbols as in Figure 2, the solid columns indicate the amounts of TXA_2 obtained when the agonists were injected to lungs perfused for 10 min with nordihydroguaiaretic acid 0.01 mM, except in the case of arachidonic acid (crossed columns), when 0.03 mM was used.

Table 3 Drug interference with the effects of aerosolized Paf-acether

Treatments	*Bronchoconstriction* (cmH_2O)	*Reduction in arterial blood pressure* (cmHg)
Controls (9)	9.3 ± 2	3.5 ± 0.4
Bilateral vagotomy (3)	9.7 ± 1.2	4.1 ± 0.4
Aspirin, 20 mg kg^{-1} (7)	2.6 ± 0.4*	0.8 ± 0.5
Aspirin 20 mg kg^{-1} + bilateral vagotomy (3)	1.8 ± 0.6*	0*
Mepyramine and methysergide 0.2 mg kg^{-1} (8)	10.3 ± 1.8	2.6 ± 0.9
Mepyramine and methysergide 0.2 mg kg^{-1} + bilateral vagotomy (5)	10.3 ± 2.1	3.6 ± 1.2
Mepyramine and methysergide 0.2 mg kg^{-1} + aspirin 20 mg kg^{-1} (5)	2.4 ± 0.93*	0*
Anti-platelet serum 0.5 ml kg^{-1} (4)	8.5 ± 2.3	2.6 ± 1.2
Prostacyclin 10 μg kg^{-1} min^{-1}, for 2 min (5)	11.8 ± 0.8	NM

NM = not measured, because of direct effects of prostacyclin; $*P < 0.01$ *In vivo* bronchoconstriction and the reduction of arterial blood pressure are shown for control animals and for those pretreated as indicated. Number of animals in parentheses.

for 15–20 min and was accompanied by systemic hypotension (Table 3). The first exposure to Paf-acether led to partial desensitization to a second exposure attempted 1–2 h later, unlike the reproducible BC following intravenous injections. Arterial blood samples collected before and at intervals during and after the aerosol showed no significant variation in platelet count, despite the pronounced BC. In 5 animals, aspirin (20 mg kg^{-1}) was given (i.v.) 10 min before the aerosol. Table 3 shows that BC was inhibited by aspirin whereas the addition of mepyramine and methysergide (0.2 mg kg^{-1} of each, given i.v. 5 min before the aerosol.) was ineffective. Furthermore, the administration of prostacyclin (10 μg kg^{-1} min^{-1}, i.v. for 2 min), an inhibitor of platelet aggregation, was inactive against the aerosol. Platelet depletion with anti-platelet serum also failed to inhibit the BC (Table 3). This table also shows that bilateral vagotomy alone or combined with mepyramine and methysergide failed to reduce BC by Paf-acether. Finally, the reduction in arterial blood pressure following the Paf-acether aerosol was suppressed by aspirin, but persisted after vagotomy or injections of mepyramine and methysergide.

Effects of Paf-acether on isolated lung strips

Lung strips were incubated in an organ bath containing 16 ml Krebs in the presence or absence of the inhibitors indicated in the Methods (5 experiments of each). Paf-acether, in concentrations of 10^{-8} M and above induced a contraction equivalent to that due to 5-HT 10^{-5}. Despite several washes started 10 min after the addition of Paf-acether, relaxation of this contraction was very slow. When the tone of the strip had returned halfway towards the resting tone (45–60 min later) further additions of Paf-acether were ineffective. Since Paf-acether might desensitize

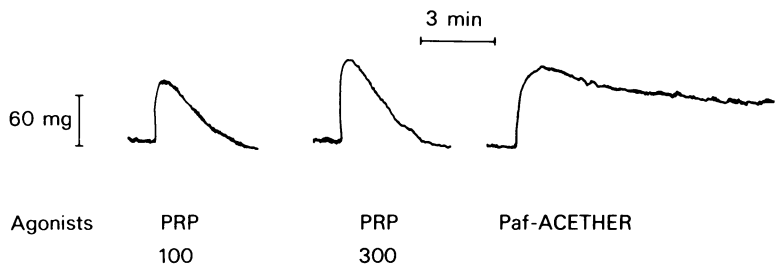

Figure 7 Contraction of the isolated lung strip induced by Paf-acether. The contractile activity of Paf-acether superfused onto a guinea-pig isolated lung strip is shown, as compared to the effect of thromboxane A_2 (guinea-pig PRP incubated with 0.1 and 0.3 mM of arachidonic acid, as indicated, and tested within 2 min). Horizontal scale: 3 min; vertical scale: 60 mg of lung contraction.

to itself during the first lung exposure, it was also assayed on lung strips superfused at 10 ml min^{-1}, as described for studying the lung outflow. Similarly, the addition of 1–10 μg of Paf-acether to the tissue was followed by a contraction which relaxed slowly (Figure 7). Exposure to a second dose of Paf-acether was never followed by renewed contractions.

Discussion

Two major points are raised by our results: (a) the mechanism of the Paf-acether-induced release of TXA_2 and of the accompanying BC and (b) the relevance of our *in vitro* results for the *in vivo* effects of Paf-acether and consequently for the pathophysiological conditions in which Paf-acether may be involved (Vargaftig, *et al.*, 1981; Benveniste & Vargaftig, 1983).

Mechanism of the in vitro *Paf-acether-induced bronchoconstrictian and release of thromboxane A_2*

Paf-acether induced BC and release of TXA_2 from the guinea-pig isolated lungs in concentrations similar to those of LTC_4 and less than those required for Bk or the TX precursor, arachidonate. The activity of Paf-acether involves specific mechanisms since neither its major pulmonary metabolite lyso-Paf-acether (Rotilio *et al.*, 1983) nor its unnatural enantiomer mimicked its bronchial and TXA_2-releasing effects. TXA_2 activity results from the administration or the phospholipase-mediated release of arachidonic acid (Vargaftig & Dao, 1971; Blackwell *et al.*, 1978). This is in agreement with the effectiveness of mepacrine, a recognised inhibitor of phospholipase A_2 (Vargaftig & Dao Hai, 1972; Damas & Bourdon, 1975), against the release of TXA_2, by Bk, LTC_4 (Omini *et al.*, 1981; Sirois *et al.*, 1982) and Paf-acether. Mepacrine is not, however, a specific phospholipase inhibitor, and its activity should be interpreted with caution. Experiments were thus performed using *p*-bromophenacyl bromide, another phospholipase inhibitor (Volwerk *et al.*, 1974) which blocks platelet activation involving phospholipase A_2 (Vargaftig, 1977) and shows *in vivo* anti-inflammatory effects (Vallee & Delahayes, 1978). *p*-Bromophenacyl bromide inhibited only Bk, suggesting that Paf-acether and LTC_4 may trigger the release of TXA_2 by a phospholipase A_2-independent mechanism since all known phospholipase A_2s bear the active arginine residue with which *p*-bromophenacyl bromide interacts (Volwerk *et al.*, 1974; Vensel & Kantrowicz, 1980). Alternatively, the cells from which arachidonate is released may differ, in that only those which are activated by Bk are readily accessible to *p*-bromophenacyl bromide.

It has been shown by Voelkel *et al.*, (1982) that Paf-acether releases leukotrienes from perfused rat lungs. Since the lipoxygenase inhibitor diethylcarbamazine, but not indomethacin, suppressed the accompanying vasoconstriction and oedema induced by intrapulmonary Paf-acether, they concluded that leukotrienes may be partly responsible for the action of Paf-acether. Our results do not support these contentions for guinea-pig lungs, since aspirin not only suppressed, as expected, the formation of TXA_2, but also blocked the accompanying BC. In this respect, the *in vitro* pulmonary effects of Paf-acether differ from those observed with platelets, which fully aggregate to Paf-acether in the presence of cyclo-oxygenase inhibition (Cazenave *et al.*, 1979; Vargaftig *et al.*, 1980). Inhibition of pulmonary formation of TXA_2 resulted in total suppression of BC supporting a causal relationship between both.

The possibility that leukotrienes may contribute to the effects of Paf-acether could not be ruled out since their bronchial effects are also inhibited by aspirin in the guinea-pig (Berry & Collier, 1964; Engineer *et al.*, 1977; Vargaftig *et al.*, 1981; Omini *et al.*, 1981; Piper, 1983). However, the ability of the leukotriene antagonist FPL 55712 to suppress the release of TXA_2 induced by LTC_4 but not Paf-acether, Bk and AA, excluded LTC_4 and LTD_4 as possible mediators of the Paf-acether response but not LTB_4 whose myotropic effects are not inhibited by FPL 55712 but are suppressed by aspirin and mepacrine (Sirois *et al.*, 1982). In addition, LTB_4-induced myotropic activity is subject to tachyphylaxis (Sirois *et al.*, 1982) as was that of Paf-acether. Nordihydroguaiaretic acid, an inhibitor of lipoxygenase (Tapel *et al.*, 1953) was used to prevent leukotriene formation. At 0.01 mM it partially reduced TXA_2 formation by Bk, LTC_4 and Paf-acether. However, at only 0.03 mM nordihydroguaiaretic acid also reduced the generation of TXA_2 directly from AA, suggesting that cyclo-oxygenase or thromboxane synthetase had also been inhibited. Nordihydroguaiaretic acid could not therefore be used as a specific lipoxygenase inhibitor. Although lungs desensitized to Paf-acether retained their capacity to release TXA_2 when stimulated with Bk or LTC_4, only studies with lungs desensitized specifically to LTB_4 (LTB_4 and LTC_4/D_4 do not cross desensitize; Sirois *et al.*, 1982) will determine if LTB_4 is involved with mediating the cyclo-oxyganase dependent effects of Paf-acether.

Guinea-pig lungs are particularly efficient in forming TXA_2 from exogenous arachidonic acid (Al-Ubaidi & Bakhle, 1979; 1980; Ally *et al.*, 1982). The rapidity with which TXA_2 activity is collected in lung perfusates after the injection of AA, and the fact that lung strips favour TXA_2 production, whereas trachea favour PGE_2 and/or PGI_2 (Grodzinska *et al.*, 1976; Mitchell & Denborough, 1980) suggest that the

synthesis of TXA_2 takes place relatively proximal to the vessels. However, since a variety of lung cells synthesize TXA_2 (Franson *et al.*, 1973; Sahu & Lynn, 1977; Yoneda, 1978; Hopkins *et al.*, 1978; Feinmark & Bailey, 1982; Crutchley *et al.*, 1983) at this stage it is impossible to define the target cell for the TX-relating agents from the whole lung.

Relevance of in vitro *bronchoconstriction for* in vivo *effects of Paf-acether*

The *in vitro* aspirin-sensitive effects of Paf-acether are apparently not related to the *in vivo* effects, which are refractory to cyclo-oxygenase inhibitors (Vargaftig *et al.*, 1980). The aspirin-resistant effects are observed when Paf-acether is given intravenously, whereas we have now shown that aspirin inhibits BC resulting from an aerosol of Paf-acether. Bronchoconstriction triggered by this aerosol is platelet-independent since neither platelet depletion nor PGI_2 administration protected against BC. This is in contrast to the effects of i.v. administration and suggests that cells located nearer to the vascular lumen are the target for Paf-acether. Hence intra-arterial injections of Paf-acether to perfused lungs induce a response similar to the aerosol *in vivo* but not i.v. administration. Paf-acether induces marked lung oedema (visual inspection) which results in protracted and irreversible BC (Figure 1). It also increases the vascular permeability (Vargaftig & Ferreira, 1982) and it is likely that by doing so it reaches the same target cells that are stimulated with the aerosol.

Paf-acether release of TXA_2 from guinea-pig isolated lungs provides a useful model to study the *p*-bromophenacyl-bromide-resistant effects and to gain insight into the effects resulting from the aerosol.

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