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Histamine and leukotriene-independent guinea-pig anaphylactic shock unaccounted for by Paf-acether

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1 Ovalbumin induced dose-dependent contractions of lung parenchyma strips from sensitized guinea-pigs, whereas Paf-acether (1-alkyl-2-acetyl-sn-glycero-3-phosphoryl choline), a potential mediator of immediate hypersensitivity, induced a single contraction, followed by specific desensitization to a second exposure.

2 Lung strips desensitized to Paf-acether contracted to ovalbumin as did non-desensitized controls, even in the presence of inhibitors of other mediators of anaphylaxis.

3 Contractions by Paf-acether and by ovalbumin were reduced by nordihydroguaiaretic acid (NDGA) and by the phospholipase A_2 inhibitor *p*-bromophenacyl bromide (0.1–0.3 mM). Three other anti-lipoxygenase agents (diethylcarbamazine, 5 mM; eicosatetraynoic acid and BW755c, 0.1 mM), reduced the contractions by ovalbumin but also those due to acetylcholine, indicating non-specific effects. Neither the anti-allergic compound sodium cromoglycate (3 mM) nor the anti-leukotriene agent, FPL 55712 (0.01 mM), inhibited the contractions by ovalbumin or by Paf-acether.

4 A sensitized strip stimulated with ovalbumin released substances which contracted a non-sensitized strip mounted in the same organ bath. The contractions of the non-sensitized strip were abolished by FPL 55712 (0.01 mM), by NDGA and BW755c, (0.1 mM), whereas those of the sensitized one were unaffected. Leukotrienes are formed by the lung strips during shock but alone, they do not explain the contractile activity.

5 The intravenous administration of ovalbumin (1 mg kg^{-1}) led to bronchoconstriction and thrombocytopenia, which were not modified by the anti-leukotriene substance FPL 55712 nor by aspirin. Bronchoconstriction was suppressed if FPL 55712 was used in combination with aspirin (20 mg kg^{-1}), mepyramine and methysergide (200 μg kg^{-1} of either). Pretreatment of the guinea-pigs with propranolol reduced this inhibition to approximately 60%. In no instance was thrombocytopenia prevented.

6 *In vitro* contractions of the actively sensitized lung strip are not fully accounted for by histamine, FPL 55712-inhibitable leukotrienes or Paf-acether, whereas in systemic anaphylaxis histamine and leukotrienes (inhibited respectively by mepyramine and by FPL 55712) have a significant role.

Introduction

Bronchoconstriction of anaphylactic shock in the guinea-pig is commonly used in the search for anti-asthma drugs, even though the mechanisms accounting for anaphylaxis and for asthma are known to differ (Austen & Orange, 1975). Slow-reacting substance of anaphylaxis may participate in asthma and since its major components are the leukotrienes C_4 and D_4 (LTC4 and LTD4) (Bach *et al.*, 1979; Morris *et al.*, 1981), their administration is used to imitate part of bronchial asthma. Investigations concerning the relevance of the leukotrienes are hindered by difficulties in devising a model for asthma. Since we showed that Paf-acether (platelet-activating factor, 1-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine) is mainly a platelet-dependent bronchoconstrictor agent (Vargaftig *et al.*, 1980), this phospholipid was added to the list of potential mediators of hypersensitivity (Vargaftig & Ferriera, 1981; Morley *et al.*, 1983). Paf-acether is released into the rabbit circulation during anaphylactic shock (McManus *et al.*, 1979), but its potential role as a mediator was not investigated. We have now studied the effects of Paf-acether on the guinea-pig lung parenchyma strip preparation and compared the responses to those triggered by antigen. We took

advantage of the fact that a single exposure to Paf-acether is followed by a contraction and by a specific desensitization to a subsequent exposure to Paf-acether itself (Findlay *et al.*, 1981; Stimler *et al.*, 1981; Lefort *et al.*, 1984; present results.). We also studied the interference of some inhibitors of the formation and/or of the effects of the leukotrienes with shock *in vivo* and with the effects of Paf-acether and of antigen on the lung parenchyma strip. Evidence is provided that a non-histamine component of active anaphylaxis is not accounted for by the formation of leukotrienes and of Paf-acether and that additional factors, apart from those described (Piper & Vane, 1969; Nijkamp *et al.*, 1976) may be important in models for immediate hypersensitivity.

Methods

Sensitization procedure

Hartley guinea-pigs of either sex (300–500 g) were injected subcutaneously with 0.5 ml of 0.9% w/v NaCl solution (saline) containing 10 μg of ovalbumin dispersed in 1 mg of $Al(OH)_3$ (modified from Andersson & Bergstrand, 1981). This injection was repeated after 14 days and the animals were used 21–27 days after the first injection. The intraperitoneal injection to naive guinea-pigs of serum prepared from the sensitized animals resulted in the transfer of the ability to undergo bronchoconstriction upon the injection of ovalbumin, for at least 14 days (unpublished).

Preparation of the isolated lung strips

The carotid artery and jugular vein of guinea-pigs anaesthesized with sodium pentobarbitone (40 mg kg^{-1} i.p.) were cannulated. Fifteen min after the intraperitoneal injection of 500 units of heparin a mid-thoracotomy was performed. The lungs were removed en masse and rinsed with 5 ml of Krebs solution injected through the pulmonary artery. Peripheral sub-pleural lung strips (2.5–3 cm long; 3–4 mm wide), dissected and mounted under a tension of 2 g, were either superfused at 8–10 ml min^{-1} or placed in an organ bath containing 16 ml of Krebs solution, aerated with 95% O_2 and 5% CO_2. Unless otherwise stated, the Krebs solution contained the various inhibitors listed below. The strips were challenged with Paf-acether or with ovalbumin, as indicated in the Results sections. When the effects of the inhibitors were studied, the responses to acetylcholine (ACh)

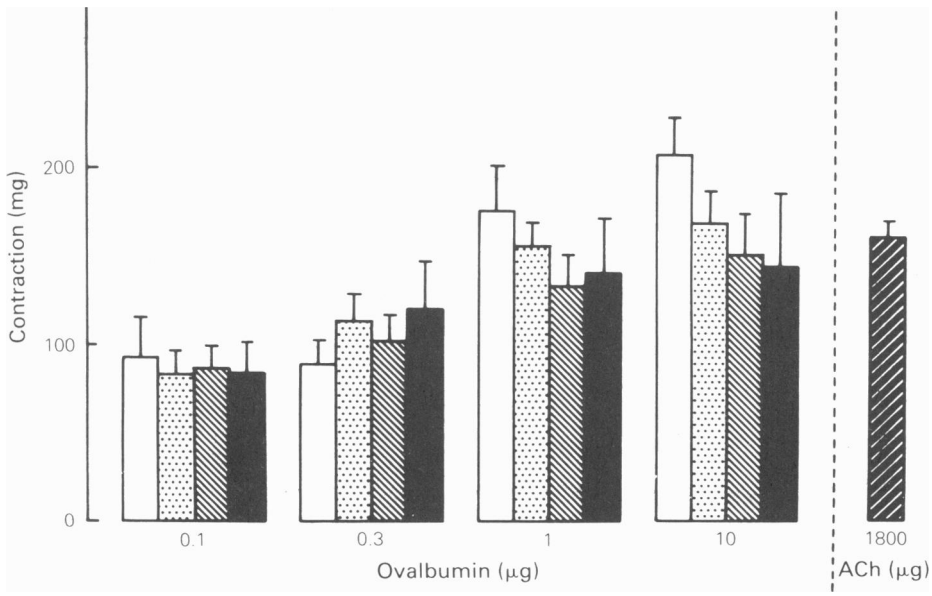

Figure 1 Concentration-dependence of the ovalbumin-induced contractions of sensitized lung parenchyma strips. Four superfused lung parenchyma strips dissected from the same sensitized animal were challenged with the indicated concentrations of ovalbumin at 30 min intervals. Open columns: Krebs solution with no added inhibitors; stippled columns: the inhibitors listed under Methods were added to the Krebs solution; hatched columns: all inhibitors present except mepyramine; closed columns: all inhibitors present except indomethacin. The effects of acetylcholine (ACh) 1800 μg) are indicated by the height of the column at the right side of the figure. Vertical scale: active contraction of the lung strips (mg, vertical lines show s.e.mean. ($n = 9$).

were determined before and after the addition of the potential antagonists, as a control for non-specific effects. Acetylcholine was tested cumulatively (18–1800 ng final concentrations added to the organ bath at 2 min intervals), or as single injections onto the superfused strips (5.4–180 μg and 1800 μg for maximal contraction).

In vivo *studies with Paf-acether given as an aerosol or by intravenous injection*

Pentobarbitone-anaesthesized guinea-pigs were prepared for the recording of bronchial resistance to inflation, as described by Lefort & Vargaftig (1978). Bronchial reactivity was checked with 5-hydroxytryptamine (5-HT, 2–4 $\mu g\,kg^{-1}$, i.v.). In some instances the animals were pretreated with propranolol (1 $mg\,kg^{-1}$, i.v. plus 3 $mg\,kg^{-1}$, i.p.). An aerosol of Paf-acether was created for 2 min with a medical aerosolator apparatus (Générateurs d'aérosol Super Marion, 13–69, typed DE) at a concentration of 300 $\mu g\,ml^{-1}$ of Paf-acether in the reservoir. This induced a bronchoconstriction equivalent to that due to intravenous 5-HT (Lefort *et al.*, 1984). Paf-acether was also administered by intravenous injections at 30 $ng\,kg^{-1}$ in the absence of propranolol. In both instances, the lungs were removed for the dissection of the strips, 30 min after inducing bronchoconstriction.

Solutions and reagents

The Krebs solution had the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2.2H_2O$ 2.5, $MgSO_4.7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 5.6. It contained the following inhibitors: indomethacin (3 μM), methysergide (0.5 μM), mepyramine (1 μM), phenoxybenzamine (0.3 μM) and propranolol (7 μM).

Materials

The following drugs were used: pentobarbitone (Nembutal, Lathévet, France); 5-hydroxytryptamine (serotonin), indomethacin, nordihydroguaiaretic acid (NDGA), phenoxybenzamine, acetylcholine (ACh), prostaglandin E_1 (PGE_1), ovalbumin, bovine serum albumin (BSA) (Sigma); $Al(OH)_3$, perchloric acid (Merck); mepyramine maleate (Rhone-Poulenc); methysergide-hydrogen-maleinate (Sandoz); propranolol (ICI); aspirin (Aspégic, Labs. Egic); heparin (Choay); sodium cromoglycate, FPL 55712, (a gift from M. Sheard, Fisons, U.K.) *p*-bromophenacyl bromide (Fluka); eicosatetraynoic acid (ETYA, Roche); BW755c (a gift from Dr S. Moncada; The Wellcome Research Labs.); LTC_4 (a gift from Dr J. Rokasch, Merck-Frost, Montreal and from Dr I.

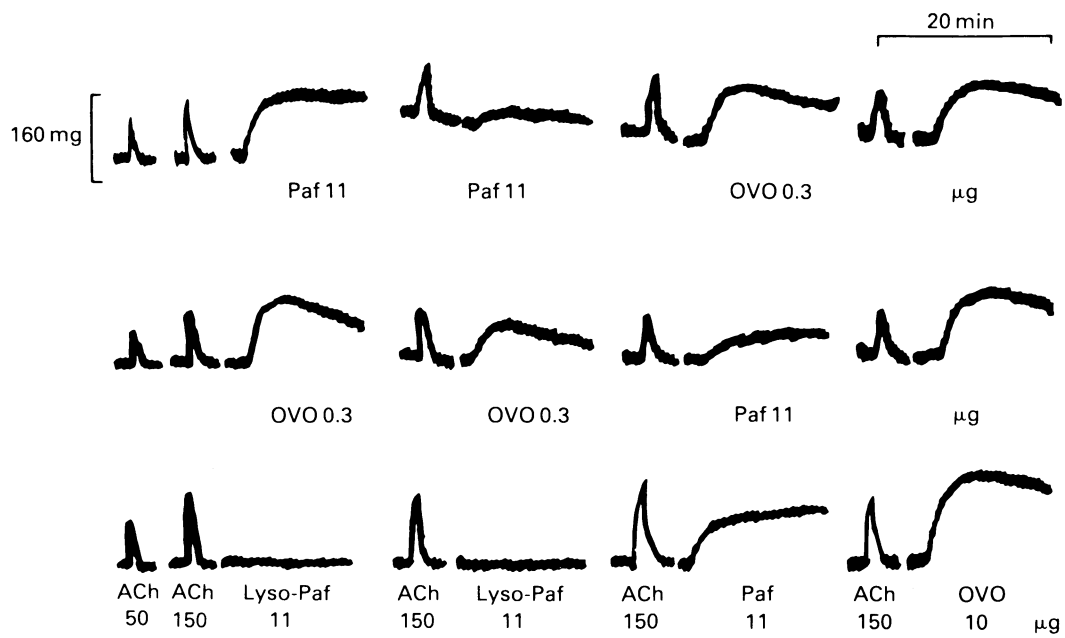

Figure 2 Effects of ovalbumin (OVO), acetylcholine (ACh), Paf-acether (Paf) and lyso-Paf-acether (Lyso-Paf) on the lung parenchyma strip. The indicated agents were applied to three superfused sensitized guinea-pig lung strips. Vertical scale: active contraction (mg); time scale: 20 min.

Stadler, Chinoin, Budapest). Paf-acether was provided by Prof. J.J. Godfroid (Université de Paris VII).

Results

Effects of antigen on the isolated tissues

Contractions of the lung strips induced by 0.1, 0.3, 1, and 10 μg ovalbumin superfused at 30–45 min intervals were dose-dependent (Figure 1), whereas when the same concentrations were repeated, the responses were somewhat reduced (Figure 2). A very high concentration of ACh was needed to induce contractions of a comparable intensity (Figure 1), which faded rapidly, whereas those due to ovalbumin were more prolonged (Figure 2).

Effects of Paf-acether on the isolated lung strip

As seen in Figures 2 and 3, 1–11 μg of Paf-acether induced a marked contraction of the lung strip, which was slow to return to baseline. Exposure to a second dose of Paf-acether was ineffective. Figure 2 also shows that lyso-Paf-acether, the deacetylated product of Paf-acether, was inactive as a contracting agent and failed to interfere with the effects of Paf-acether. When the latter was tested on a strip collected from an animal pretreated with it intravenously and/or by inhalation, the *in vitro* responsiveness was maintained (not shown).

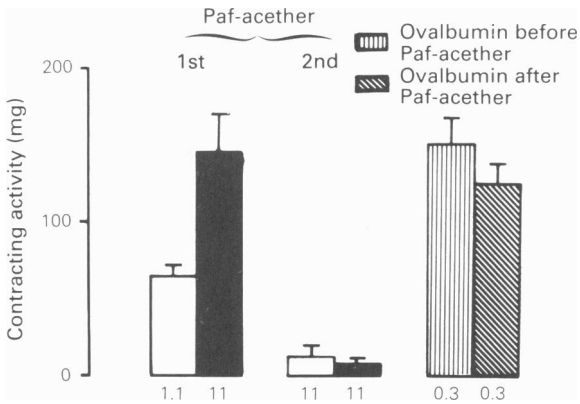

Figure 3 Failure of desensitization to Paf-acether to interfere with the effects of ovalbumin. A first exposure (1st) of superfused guinea-pig lung strips to Paf-acether (1.1 or 11 μg) was followed, 3 h later, either by a second exposure (2nd) to 11 μg of Paf-acether itself, or to ovalbumin (0.3 mg). Open columns: contraction of the same strip to 1.1 ($n = 5$) and then to 11 μg of Paf-acether.

Interactions between anaphylactic shock and Paf-acether

The responsiveness to ovalbumin was maintained when the tissues were desensitized to Paf-acether (Figures 2 and 3). In 4 experiments Paf-acether alone contracted the tissues by 48 ± 14 mg, whereas when it followed exposure to ovalbumin the response was reduced to 28 ± 8 mg (NS).

Interference of potential inhibitors with the effects of antigen and of Paf-acether on lung strips

NDGA superfused for 10–60 min onto the lung strips at final concentrations of 0.1–0.3 mM, inhibited by 50–100% the contractions induced by Paf-acether (Table 1). At 0.1 mM, NDGA blocked those contractions when the tests were performed in the isolated organ bath, whereas 0.03 mM was inactive (Table 1). The contracting effects of superfused antigen were also suppressed by 0.1 mM of NDGA. At this concentration NDGA inhibited by 39% and 66% the effects of ACh, superfused at 540 μg, or added to the organ bath at 0.3 μg, respectively. Contractions by LTC_4 superfused at 0.5 nM to 0.02 μM were also inhibited by NDGA, suggesting non-specific effects. Compounds BW755c and ETYA added to the organ bath at 0.1 mM were inactive against Paf-acether. At those concentrations, both antagonists were partially effective against antigen (Table 1). The anti-phospholipase A_2 compound bromophenacyl bromide at 0.03 mM in the isolated organ bath or at 0.1 mM in superfusion, blocked the contractions to Paf-acether (Table 1). At 0.3 mM,bromophenacyl bromide strongly inhibited the contractions to ovalbumin, but the effects of superfused ACh were also reduced. Since it was difficult to discriminate between the direct effects of bromophenacyl bromide, and those that might be attributed to inhibition of phospholipase A_2, experiments were performed in which bromophenacyl bromide was not administered directly to the assay tissues but was infused into the pulmonary artery for 10 min at a final concentration of 0.4 mM before dissecting the lung strip. Under these conditions, control strips challenged withy 0.9 μg of Paf-acether in the superfusion fluid or 10 μg in the organ bath contracted by 113 ± 27 mg and 59 ± 12 mg, respectively, whereas the strips collected from bromophenacyl bromide-treated animals contracted by 103 ± 22 mg and 39 ± 10 mg, respectively ($n = 4$, $P > 0.05$). Contractions due to the smallest concentration of ovalbumin (0.1 and 0.3 μg) were not statistically different when the strips were collected from bromophenacyl bromide-treated lungs as compared to controls. In contrast, the contractions induced by 1 and 10 μg ovalbumin were blocked by more than 50%

Table 1 Inhibition (%) of the contractile effects of ovalbumin, Paf-acether and acetylcholine

Potential inhibitor		*Ovalbumin (μg)* (superfusion)			*Paf-acether (μg)* (superfusion)	*Paf-acether (μg)* (organ bath)	*Acetylcholine (μg)* (superfusion)	*Acetylcholine (μg)* (organ bath)
	mM	0.3	1	10	(1.1)	(0.9)	(540)	(0.3)
1 *Anti-lipoxygenase:*								
NDGA	0.3	—	—	—	100	—	66 ± 5.5	—
	0.1	100	100	100	58.6 ± 11.2	100	39 ± 6.3	66 ± 16
	0.03	74 ± 9	80 ± 6.6	52 ± 7.5	0	9.5	0	0
ETYA	0.1	68 ± 8	53.5 ± 11	41.4 ± 8	—	29.2 ± 18	—	55 ± 1.3
BW755c	0.1	53 ± 20	39 ± 17.4	30 ± 10	—	22.2 ± 10	—	28.8 ± 1.3
Diethyl-	5	—	64 ± 12	48 ± 13	—	—	21 ± 7	—
carbamazine	0.5	—	11 ± 11	14 ± 11	—	—	0	—
2 *Anti-phospholipase A2:*								
BPB	0.3	75 ± 13	79 ± 11	41 ± 15	—	—	51.2 ± 5.2	—
	0.1	—	—	—	96.5 ± 3.5	—	8.3 ± 3.59	—
	0.03	—	—	—	0	92 ± 4.6	—	28.7 ± 6.3
	0.01	—	—	—	—	51 ± 10.5	—	35 ± 16
3 *Anti-leukotriene*								
FPL 55712	0.01	17 ± 10	0	0	—	0	—	0
4 *Anti-allergic*								
Cromoglycate	3	14.75 ± 14	7 ± 3.4	0	—	0	—	29.5 ± 5.5

The indicated drugs were tested against ovalbumin on sensitized guinea-pig lung strips (in superfusion), or against Paf-acether and ACh (in superfusion and in an organ bath). In each case, four lung strips were dissected; one was used as a control, and the others were treated with the potential inhibitors. The results are expressed in % inhibition ± s.e.mean, the responses of treated strips being compared to those of the control strips ($n = 3-8$).

in 3 experiments, and unaffected in the remaining three. Finally, neither compound FPL 55712 nor sodium cromoglycate (10 μM and 3 mM, respectively) was effective when tested against the contractions due to Paf-acether superfused or added to the organ bath and against superfused ovalbumin. This concentration of FPL 55712 suppresses the effects of LTC_4 (0.01 μM) (not shown).

Attempts to antagonize anaphylactic shock in vivo *with FPL 55712*

Bronchoconstriction and thrombocytopenia (which amounted to 86 ± 4.5% within 6 min) followed the intravenous perfusion of ovalbumin (1 mg kg^{-1} for 2 min) to the guinea-pigs. The combination of aspirin (20 mg kg^{-1}, i.v.), mepyramine and methysergide (0.2 mg kg^{-1} of each), which suppresses bronchoconstriction by Paf-acether in propranolol-treated animals (Vargaftig *et al.*, 1982) failed to block bronchoconstriction induced by shock under similar conditions (Figure 4). The anti-leukotriene substance, FPL 55712 (10 mg kg^{-1}), used alone was inactive but it blocked approximately 60% of the bronchoconstriction when associated with aspirin, mepyramine and methysergide ($P < 0.05$). In non-propranolol-treated animals, FPL 55712 alone was also inactive, but bronchoconstriction was practically suppressed when it was associated with aspirin, methysergide and mepyramine (Figure 4). Finally when these three drugs were used alone, bronchoconstriction was blocked by approx. 40% ($P < 0.05$); omission of aspirin did not modify these effects. In no instance was thrombocytopenia modified by the various drug combinations.

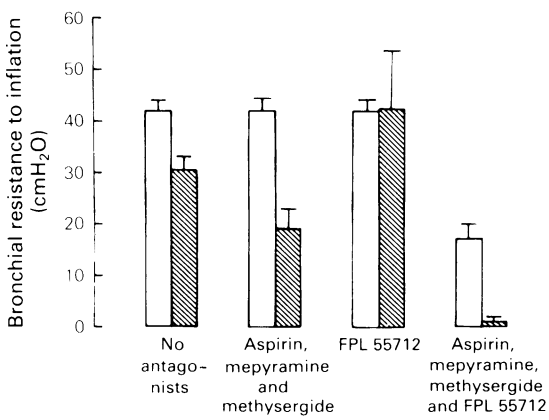

Figure 4 Interference of potential antagonist with shock induced in control and in propranolol-treated sensitized guinea-pigs. Ovalbumin was perfused i.v. at 1 mg kg^{-1} for 2 min to sensitized guinea-pigs, 10 min after treatment with aspirin (20 mg kg^{-1}), mepyramine (200 μg kg^{-1}) and methysergide (200 μg kg^{-1}). FPL 55712 (10 mg kg^{-1}) was perfused i.v. during 4 min, starting 1 min before ovalbumin. The maximal bronchial resistance to inflation (vertical scale in cmH_2O) is indicated by the height of the columns (open: animals pretreated with propranolol; hatched: no propranolol). The various treatments are indicated below each pair of columns and the doses are given in the text ($n = 3-5$).

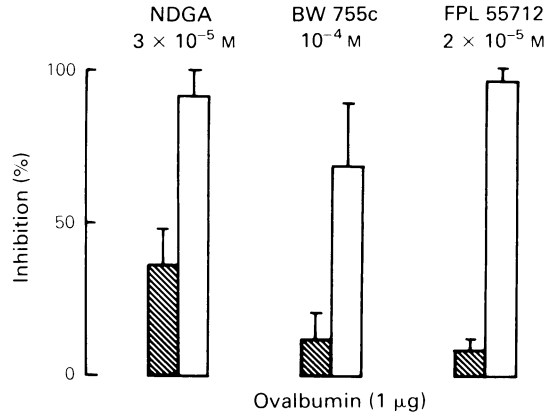

Figure 5 Failure of NDGA, of BW755c and of FPL 55712 to prevent the antigen-induced contractions of the sensitized donor lung strips, when blocking the contractions of the non-sensitized recipient strips. Lung strips from a sensitized and from a non-sensitized animal were paired and challenged simultaneously with ovalbumin (1 μg). The height of the columns indicates the % inhibition of the contractions of the donor sensitized (hatched columns) or of the recipient non-sensitized strips (open columns) in the presence of NDGA (30 μM), BW755c (100 μM) or FPL 55712 (20 μM); ($n = 4-5$); vertical lines show s.e.mean.

Evidence for the participation of non-leukotriene mediators in shock

The above results suggested that, during anaphylactic shock, mediators other than histamine and the leukotrienes and which do not cross-desensitize with Paf-acether, may play a role. To check for this possibility, two strips, one from a sensitized (donor strip) and another from a non-sensitized guinea-pig (recipient strip), were placed together in the organ bath. When ovalbumin was added to the bath, the donor strip contracted and this was followed, within approximately 30 s, by the contraction of the recipient strip. When FPL 55712 was added before ovalbumin at 0.01 mM, the contractions of the donor strip were unaffected, whereas those of the recipient strip were suppressed (Figure 5). The anti-lipoxygenase compounds BW755c and NDGA, added to the bath at 0.1 mM and 0.03 mM respectively, inhibited the contractions of the recipient strip and failed to interfere with those of the

donor strip (Figure 5). Finally, when FPL 55712 was added at the height of the contraction, the recipient strip relaxed, whereas the contraction of the donor strip persisted (not shown).

Discussion

Paf-acether, a potential mediator of inflammation and/or hypersensitivity, induces acute inflammation (Stimler *et al.*, 1981; Wedmore & Williams, 1981; Bonnet *et al.*, 1981; Vargaftig & Ferreira, 1981; Humphrey *et al.*, 1982; Page *et al.*, 1982; 1983; Morley *et al.*, 1983), platelet-dependent and cyclo-oxygenase-independent bronchoconstriction in the guinea-pig (Vargaftig *et al.*, 1980) and contracts the guinea-pig and rabbit lung strips (Stimler *et al.*, 1981; Camussi *et al.*, 1983). Since Paf-acether triggers the release of leukotrienes from rat lungs (Voelkel *et al.*, 1982) and from rabbit neutrophils (Lynch *et al.*, 1979), it seemed reasonable to investigate whether its ability to stimulate lung parenchymal strips would be modified by agents which block the generation or the activity of the leukotrienes. Assays were performed in the presence of mepyramine and indomethacin, in order to rule out the role of histamine and cyclo-oxygenase products respectively. Neither BW755c nor ETYA, at concentrations which block the lipoxygenases (Hitchcock, 1978; Burka & Flower, 1979; Orning & Hammarstrom, 1980; Armour *et al.*, 1981; Piper & Temple, 1981; Pattersson *et al.*, 1981) inhibited the effects of Paf-acether. Moreover, BW755c suppressed the generation of leukotriene-like (i.e., FPL 55712 – inhibitable) substances detected with the coupled strips, validating its use as an inhibitor of the formation of leukotrienes in our system. NDGA was effective against Paf-acether in the organ bath and in superfusion, but it also reduced the effects of ACh, even though to a lesser extent than those of Paf-acether. Since indomethacin was present in the bathing solution, the anti-cyclo-oxygenase activity of NDGA (Lefort *et al.*, 1984) cannot account for inhibition of Paf-acether, but lipoxygenase products might do so (Burka & Saad, 1984). Nevertheless, since the contractile effects of Paf-acether were not blocked by compound FPL 55712, in accordance with the demonstration that the latter fails to prevent the Paf-acether-induced release of thromboxane A_2 from guinea-pig lungs (Lefort *et al.*, 1984), formation of LTC_4 and LTD_4 cannot explain those effects. The possible mediation of the effects of Paf-acether by non-leukotriene lipoxygenase products, accounting for the effects of NDGA, was not ruled out, but is unlikely since the anti-lipoxygenase substances BW755c and ETYA failed to imitate NDGA.

Disodium cromoglycate (DSCG) was completely inactive against the effects of Paf-acether *in vitro*, in accordance with *in vivo* results (Lewis *et al.*, 1984), and in contrast to the inhibition described by Basran *et al.* (1983) with very high concentrations applied with Paf-acether to human skin. This indicates that the anti-allergic activity of DSCG is not accounted for by inhibition of the effects of Paf-acether.

The phospholipase A_2 inhibitor, bromophenacyl bromide, at concentrations of 0.1 mM and 0.03 mM suppressed the lung strip contractions due to Paf-acether in superfusion and in the organ bath, respectively, and did not interfere significantly with those due to ACh. This is at variance with our own findings that the contractions by Paf-acether were not inhibited when bromophenacyl bromide was injected intra-arterially before the dissection of the strip, and with those of Lefort *et al.* (1984), showing that this procedure for the injection of bromophenacyl bromide is effective in blocking the release of thromboxane A_2 by bradykinin. The discrepancy may result from the different routes of administration of bromophenacyl bromide, reaching different cell populations, particularly in the case of the lung strip, which is a very heterogeneous preparation (Songsiridej *et al.*, 1983). The different behaviour according to the routes of administration may also explain why the lung strips collected from animals pretreated with Paf-acether intraveneously and by aerosol were not desensitized, contrary to the results reported by Camussi *et al.* (1983) in which the tracheal instillation of Paf-acether to the rabbit prevented the subsequent *in vitro* contraction of the lung strip.

In vitro testing of anti-anaphylactic drugs required an appropriate model. After a few unsuccessful trials with the conventional organ bath, where a single addition of ovalbumin desensitized fully to its subsequent additions, we demonstrated that appropriate increments of the amounts of ovalbumin added to superfused lung strips result in predictable dose-dependent effects (Figure 1). In this model, neither mepyramine nor indomethacin was effective, validating its use for testing drugs expected to interfere with shock by non-histamine and non-cyclo-oxygenase mechanisms. Under these conditions, the reagents tested against Paf-acether could be assayed reliably against ovalbumin. At 0.1 mM, NDGA suppressed the ovalbumin-induced contractions, inhibition still being observed at 0.03 mM. Since indomethacin was present in the Krebs solution, here also the effectiveness of NDGA is not accounted for by its anti-cyclo-oxygenase activity in the guinea-pig lungs (Lefort *et al.*, 1984). Under our present conditions, NDGA thus behaved as an anti-anaphylactic compound, and this activity was imitated by other anti-lipoxygenase agents, i.e., ETYA, BW755c and diethylcarbamazine. This pattern of activity supports the involvement of lipoxygenase products in shock, even though these inhibitors were not specific against ovalbumin. In agreement,

bromophenacyl bromide superfused at 0.3 mM blocked 50–70% of the contractions to ovalbumin but here again, when the lungs were infused with the anti-phospholipase agent before dissecting the strips, significant inhibition was not found, suggesting non-specific effects on the contractile tissues.

FPL 55712, an end-organ inhibitor of the leukotrienes (Augstein *et al.*, 1973) also failed to inhibit the contractions of the antigen-stimulated lung parenchyma strips. The results with the coupled strips are particularly illustrative in this respect, since the contractions of the sensitized strip were not affected by FPL 55712, at concentrations that suppressed those of the recipient strips. This shows that, indeed, leukotrienes sensitive to inhibition by FPL 55712 are formed by the shocked tissue, as was demonstrated by Fleisch *et al.* (1982); however, this formation does not account for the active contractions. The anti-lipoxygenase compounds BW755c and NDGA suppressed the leukotriene-like contraction of the recipient strip, whereas that of the shocked strip remained unaffected (Fleisch *et al.*, 1982). An alternative mechanism and/or mediators for the mepyramine and FPL 55712-resistant effects of antigen is thus needed, as suggested initially by Songsiridej *et al.* (1983). Paf-acether was initially suggested as an alternative mediator, since it induces marked tonic contractions of the lung strips. As we had no end-organ inhibitor of Paf-acether, shock was triggered in desensitized strips. Under those conditions, the contractions evoked by ovalbumin were maintained, in spite of the simultaneous presence of inhibitors of other potential mediators of anaphylaxis (indomethacin, mepyramine, FPL 55712). Thus it is unlikely that the release of Paf-acether by the guinea-pig lung is the alternative mechanism accounting for the non-histamine and non-leukotriene anaphylactic lung strip contraction, even though Rotilio *et al.* (1983) detected lyso-Paf-acether, a metabolite of Paf-acether, in perfusates from shocked lungs.

FPL 55712, which also failed to inhibit bronchoconstriction of anaphylaxis in animals sensitized in a system which favours IgE-dependent reactions (Andersson & Bergstrand, 1981), synergizes with an anti-histaminic agent to inhibit shock in propranolol-treated animals sensitized with large doses of ovalbumin (Andersson *et al.*, 1983; Lewis *et al.*, 1983). Our results show that when low sensitizing doses of ovalbumin are used, FPL 55712 alone is inactive against bronchoconstriction in propranolol-treated animals but that its use in combination with mepyramine, methysergide and aspirin reduces bronchoconstriction by 60%. Bronchoconstriction was suppressed by this drug combination when propranolol was omitted. Thus, it appears that a combined treatment directed against various mediators, particularly histamine and LTC_4/LTD_4, can be effective *in vivo*, whereas lung strips collected from animals sensitized under similar conditions and tested *in vitro* are refractory to the same combined antagonists. Under sensitizing conditions which favour IgG, Collier & Shorley (1960) and Collier & James (1967) also noted that bronchoconstriction *in vivo* by antigen was lessened by an antihistamine, whereas aspirin was either inactive or only effective against an immediate component of shock. The discrepancy between the *in vivo* and the *in vitro* results with lung strips may be due to the overwhelming amounts of histamine released from the guinea-pig liver during shock, which obviously are not involved when isolated lung preparations are studied. Since antihistamines are ineffective against human bronchial asthma the *in vitro* model may be more relevant to the human situation and more useful for drug testing.

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