Limited interference of specific Paf antagonists with hyper-responsiveness to Paf itself of lungs from actively sensitized guinea-pigs

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1 The interference of various platelet-activating factor (Paf) antagonists with Paf- and antigen-induced effects in isolated lungs from actively sensitized guinea-pigs was investigated.

2 WEB 2086 and BN 52021, two antagonists structurally unrelated to Paf, at concentrations 10-100 fold above those exerting a potent and selective inhibition of the effects of Paf in lungs from non-immunized guinea-pigs, failed to inhibit bronchoconstriction and mediator release evoked by the phospholipid when administered into lungs from actively sensitized animals.

3 In contrast to WEB 2086 and BN 52021, antagonists such as Ro 19-3704 and Ro 19-1400, structurally related to the Paf molecule, at concentrations which abrogate the effects of Paf in lungs from non-immunized guinea-pigs, inhibited bronchoconstriction and release of histamine and leukotriene-like material evoked by the intra-arterial administration of Paf into lungs from actively sensitized animals.

4 Ro 19-3704 and Ro 19-1400 also inhibited markedly the release of leukotriene C₄ (LTC₄)-like material and, to a smaller extent, the histamine secretion induced by 10 µg arachidonic acid.

5 CV 6209, another Paf antagonist structurally related to the phospholipid, failed to antagonize its bronchopulmonary and secretory effects in sensitized lungs.

6 All the antagonists used, irrespective of their ability to interfere or not with bronchoconstriction and mediator release triggered by Paf, suppressed oedema formation as measured by the increase in lung wet weight induced by either Paf or ovalbumin.

7 Our results indicate that: (i) the increase in vascular permeability and the subsequent oedema formation on one hand and the bronchopulmonary effects of Paf on the other hand are mediated by different mechanisms; and (ii) active sensitization provokes a marked modification of the lung reactivity to Paf.

Introduction

It has been recently established that active sensitization of guinea-pigs leads to *in vitro* bronchopulmonary hyper-responsiveness to different agonists, including platelet-activating factor (Paf) (Pretolani *et al.*, 1988). Indeed, lungs from these animals exhibit an enhanced bronchoconstrictor response, release more cyclo-oxygenase and lipoxygenase metabolites and secrete histamine in response to Paf when compared to lungs from non-immunized guinea-pigs. This phenomenon appears to be independent of the ability of the lung to undergo anaphylaxis, since lungs from passively sensitized animals show bronchopulmonary responses and mediator release following Paf administration

similar to those observed in lungs from non-immunized guinea-pigs, even though they respond to antigen as lungs from actively sensitized animals (Pretolani *et al.*, 1988).

The differences between lungs from actively sensitized and non-sensitized guinea-pigs were further emphasized by studying the pharmacological modulation of the bronchoconstrictor and secretory effects of Paf. Indeed, bronchoconstriction induced by Paf, which is blocked by cyclo-oxygenase inhibitors when lungs isolated from non-immunized guinea-pigs are used (Lefort *et al.*, 1984), becomes refractory to aspirin or indomethacin when lungs are obtained from actively sensitized animals (Pretolani *et al.*, 1988). Furthermore, the increased bronchoconstrictor response to Paf observed in sensitized

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lungs is markedly reduced by BW 755C, a mixed cyclo-oxygenase and lipoxygenase inhibitor (Higgs *et al.*, 1979), in the presence of mepyramine, indicating that leukotrienes participate in the guinea-pig lung hyper-responsiveness to Paf.

Recently, potent and selective Paf antagonists have been described which, besides their activity against Paf in various experimental models, are claimed to inhibit allergic reactions (reviewed by Braquet *et al.*, 1987). Among these compounds, Ro 19-3704, Ro 19-1400 (Burri *et al.*, 1985) or CV 3988 (Terashita *et al.*, 1983), represent structural analogues of the Paf molecule, whereas others, such as WEB 2086 (Casals-Stenzel, 1987; 1988; Pretolani *et al.*, 1987) or BN 52021 (Braquet *et al.*, 1985), are structurally unrelated. Since active sensitization modifies the guinea-pig lung reactivity to Paf (Pretolani *et al.*, 1988), the interference of its antagonists with the bronchopulmonary and mediator-releasing effect of the phospholipid in lungs from actively sensitized guinea-pigs was now investigated. In addition, since peptido-leukotrienes are claimed to be essential for allergy (Barnes & Costello, 1986), to participate in acute lung anaphylaxis and to be released by Paf from sensitized lungs (Pretolani *et al.*, 1989), the interference of Paf antagonists with their formation was also investigated.

Methods

Sensitization procedure

Hartley guinea-pigs of either sex (400–600 g) were actively sensitized by two s.c. injections separated by two weeks of 0.5 ml of 0.9% NaCl (saline) containing 10 µg ovalbumin dispersed in 1 mg Al(OH)₃. The animals were used 7 days after the second injection. This procedure promotes the production of high titres of specific homocytotropic antibodies (Ovary, 1964). The titre, measured in the serum obtained from animals killed one week after the booster injection of the antigen, was approximately of 1/800 (Pretolani *et al.*, 1988). In some experiments guinea-pigs were used at defined time-intervals during the sensitization period; in others the booster injection was omitted and the animals were used 14 or 21 days after the first injection of the antigen. Since lungs from non-immunized or Al(OH)₃-injected guinea-pigs exhibited similar responses to Paf (Pretolani *et al.*, 1988), the former were used as controls.

Tracheal strip preparation

Non-immunized guinea-pigs were anaesthetized with sodium pentobarbitone (30 mg kg⁻¹, i.p.) and trache-

ae were removed. To increase the sensitivity of the strips to contractile agents (Hay *et al.*, 1986), the epithelium was removed by gentle rubbing of the mucosal surfaces with a cotton wool-coated probe and the tissues were cut in spirals. Each preparation was connected to a Gould Statham isometric force transducer under 2 g tension in an organ bath and superfused with a Krebs solution at 37°C containing 2.5 g l⁻¹ bovine serum albumin (Krebs-BSA) and gassed continuously with 95% O₂:5% CO₂. The Krebs-BSA solution was supplemented with phenoxylbenzamine (3 µM), mepyramine (2 µM), methysergide (0.5 µM), atropine (10 µM) and indomethacin (3 µM), to avoid contractile responses by mechanisms other than those evoked by the release of leukotriene-like material from the lung. The tissues were allowed to equilibrate for 2 h and, after this period, the stable endoperoxide analogue, U 46619 was administered at doses of 1 or 2 nmol to assess their sensitivity.

Lung perfusion experiments

Actively sensitized guinea-pigs were anaesthetized as above, tracheae were cannulated and the animals ventilated (60 strokes min⁻¹, 10 ml kg⁻¹ body weight) with a Palmer miniature respiratory pump. A thoracotomy was performed and the lungs were removed as described (Lefort *et al.*, 1984). Each organ was placed in a plastic chamber and immediately ventilated (60 strokes min⁻¹, 10 ml kg⁻¹ body weight) and perfused (10 ml min⁻¹, 37°C) with a gassed (95% O₂:5% CO₂) Krebs-BSA solution containing 3 µM indomethacin and 2 µM mepyramine. Indomethacin was used to prevent the formation of any cyclo-oxygenase metabolites which contract the tracheal strip and might interfere with the responses to leukotriene-like material from the lung. Mepyramine abolishes the histamine component in the bronchoconstrictor action of Paf in lungs from actively sensitized guinea-pigs (Pretolani *et al.*, 1988). After passage through the lung, the perfusion solution was directed to the tracheal strip and the superfusion system for the latter organ was stopped. Thus, the solution eluting from the lung was used to perfuse the tracheal strip. After a 10 min period for equilibration, increasing doses (1 and 100 ng in 100 µl of saline containing 0.1% BSA) of Paf were injected into the pulmonary artery at 10 min intervals. Bronchoconstrictor responses were recorded by a pressure transducer located between the trachea and the respiratory pump and expressed as percentages of increase, calculated over the basal values measured prior to the administration of the agonists. Before the end of each experiment, 10 µg ovalbumin was administered and the lung response was recorded. Fractions of lung effluent were collected before,

during the first 3 min and between the 3rd and 6th min after the successive injections of the two doses of Paf and ovalbumin for the determination of immunoreactive leukotriene C_4 (LTC_4)-like material and histamine, as described below. In a series of experiments, arachidonic acid was injected at the dose of $10\mu g$ instead of Paf and lung response was investigated, as described above.

When the effect of the various Paf antagonists was studied, the compounds were dissolved up to the indicated concentrations in the Krebs-BSA solution used for the lung perfusion and Paf and ovalbumin were administered, as described above.

Determination of immunoreactive LTC_4 -like material and histamine

Immunoreactive LTC_4 -like material in fractions of lung perfusate was quantified by radioimmunoassay, as previously described (Aehringhaus *et al.*, 1982). Dextran-coated charcoal was used to separate unbound ligand by centrifugation at $1200g$ for 10 min at $4^\circ C$. The monoclonal anti- LTC_4 antibody employed (kindly provided by Dr U. Zor, Weizmann Institut, Rehovot, Israel) was 10% crossreactive with LTD_4 and less than 0.1% with LTA_4 and LTB_4 . The sensitivity of the assay was approximately 15 pg of immunoreactive LTC_4 per 0.1 ml sample.

For the determination of histamine, 1 ml-aliquots of the collected fractions were mixed with 1 ml of a 0.8N perchloric acid solution. After centrifugation for 10 min at $1000g$ and $4^\circ C$, the supernatants were stored at $4^\circ C$. An automatic spectrofluorometric assay for histamine was performed according to previously described methods (Shore *et al.*, 1959; Lebel, 1983).

Measurement of lung oedema formation

The increase in lung wet weight following the intra-arterial injections of Paf and ovalbumin into lungs from actively sensitized guinea-pigs was used to evaluate the oedema formation. Lungs were weighed before, after the administration of 1 and 100 ng Paf and at the end of the experiments, after the injection of $10\mu g$ ovalbumin in the absence and in the presence of the various Paf antagonists. The increases in lung weights were compared to those of lungs perfused for 40 min and injected at 10 min intervals with 0.1 ml of the Paf vehicle, i.e. saline containing 0.1% BSA.

Materials

The composition of the Krebs solution was (in mM): NaCl 118, KCl 4.7, $CaCl_2 \cdot 2H_2O$ 2.5, $MgSO_4 \cdot$

$7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 5.6. The drugs used were as follows: Paf (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine), indomethacin, phenoxybenzamine, atropine sulphate, BSA fraction V (Sigma, St. Louis, MO, U.S.A.); $Al(OH)_3$, perchloric acid (Merck, Darmstadt, GFR); mepyramine maleate (Rhône-Poulenc, Vitry/Seine, France); methysergide hydrogen maleinate (Sandoz, Basel, Switzerland); pentobarbitone (sodium pentobarbital, Clin-Midy, Montpellier, France); chicken ovalbumin (Miles, Naperville, IL, U.S.A.); U 46619 (15 (S)-hydroxy-11,9 (epoxymethano)prosta-5Z, 13E-dienoic acid) was kindly provided by Dr M. Bach, Upjohn Co, Kalamazoo, U.S.A.; LTC_4 (PIRMED-CNRS, Paris, France); WEB 2086 (3-[4-(2-chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]-triazolo-[4,3-a]-[1,4]-diazepin-2-yl]-1-(4-morpholinyl)-1-propanone, from Boehringer-Ingelheim, F.R.G.), was initially dissolved in HCl 50 mM in saline and further diluted to the appropriate concentrations in saline; BN 52021 (9H-1,7a-(epoxymethano)-1H,6aH-cyclopenta[c]furo - [2,3 - b]furo - [3',2',:3,4]cyclopenta - [1,2-d]-furan-5,9,12-(4H)-trione, 3-tert-butylhexahydro-4,4b-11-tri-hydroxy-8-methyl) from Institut Henri Beaufour, Le Plessis Robinson, France), was dissolved in NaOH 0.1N and the pH was adjusted to 7.4 with HCl 0.1N; Ro 19-3704 (3-[4-[(R)-2 - [(methoxycarbonyl)oxy] - 3 - [(octadecylcarbamoyl)oxy]propoxy]butyl]thiazolium iodide and Ro 19-1400 (3-[3-[(R)-2-(methoxycarbonyl)-3 - [(octadecylcarbamoyl)oxy]propoxy]carbonyl]propyl]thiazolium hydroxide), from Roche, Basel, Switzerland) were dissolved in ethanol and further diluted in saline; CV-6209 (2-[N-acetyl-N-(2-methoxy-3-octadecyl-carbamoyloxy-propoxycarbonyl) - aminomethyl] - 1 - ethylpyridinium chloride) from Takeda Chemical Ind., Osaka, Japan, was dissolved in saline.

Statistical analysis

Results are expressed as mean $\pm$ s.e.mean of the indicated number of experiments and significance was assessed by Student's *t* test for unpaired values.

Results

Interference of the antagonists with Paf- and antigen-induced bronchoconstriction and tracheal strip contraction of lungs from actively sensitized guinea-pigs

The administration of 1 ng Paf into the pulmonary artery of lungs from actively sensitized guinea-pigs

Figure 1 Bronchoconstriction induced by intra-arterial injections of 1 ng Paf into isolated perfused lungs from actively sensitized guinea-pigs in the absence (A) and in the presence of WEB 2086 (B), BN 52021 (C), CV 6209 (D), Ro 19-3704 (E) and Ro 19-1400 (F). All drugs were dissolved in the Krebs-BSA solution at the final concentration of 10 μ M, except BN 52021, which was used at 0.1 mM. Bronchoconstriction is expressed as % increase of the bronchial resistance to inflation measured prior to the administration of 1 ng Paf. Results represent the mean of 5 to 8 experiments; vertical bars show s.e.mean. *** $P < 0.001$.

induced a maximal and irreversible bronchoconstriction (Figure 1), which prevented further responses to 100 ng of the phospholipid to be recorded. Following the triggering of bronchoconstriction,

Figure 2 Tracheal strip response (in mg tension) induced by the intra-arterial administrations of Paf (1 and 100 ng, a) and ovalbumin (Ovalb, 10 μ g, b) into isolated perfused lungs from actively sensitized guinea-pigs in the absence (A) and in the presence of 10 μ M WEB 2086 (B), 0.1 mM BN 52021 (C) and 10 μ M CV 6209 (D). Data are expressed as mean of 5 to 8 experiments; vertical bars show s.e.mean.

Figure 3 Tracheal strip response (in mg tension) induced by the intra-arterial administrations of Paf (1 and 100 ng, a) and ovalbumin (Ovalb, 10 μ g, b) into isolated perfused lungs from actively sensitized guinea-pigs, in the absence (open columns) and in the presence of Ro 19-3704 (solid columns) and Ro 19-1400 (lined columns), used at the concentration of 10 μ M. Data are expressed as mean of 5 to 8 experiments; vertical bars show s.e.mean. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

a marked contraction of the superfused tracheal strip was noted (Figure 2). When Paf was then injected into the pulmonary artery at a dose of 100 ng, only a minimal contraction of the tracheal strip was observed (Figure 2). At the end of the experiment, 10 μ g ovalbumin was injected into the lung but, due to the maximal and irreversible bronchoconstriction evoked by the previous administrations of Paf, no further increase in bronchial resistance was recorded. By contrast, the superfused tracheal strip contracted largely following the antigen challenge (Figure 2), demonstrating the presence of a spasmogenic substance in the effluent from antigen-challenged lungs, in spite of desensitization to Paf.

In the presence of Paf antagonists WEB 2086 or CV 6209 (10 μ M) or BN 52021 (0.1 mM), bronchoconstriction induced by 1 ng Paf was equivalent to controls, i.e., reached a maximal value which precluded the determination of statistical variations (Figure 1). In a separate series of experiments the effect of BN 52021, WEB 2086 and CV 6209 on bronchoconstriction and tracheal strip contraction induced by lower doses (0.01 or 0.1 ng) of Paf was investigated and, as in the case of higher doses of the phospholipid, these antagonists were ineffective against bronchoconstriction as well as tracheal strip contraction (not shown).

The amplitude of the tracheal contraction in response to the injection of 10 μ g ovalbumin into the lung was also unaffected by WEB 2086, BN 52021 or

Figure 4 Release of histamine (a) and immunoreactive leukotriene C₄ (LTC₄)-like material (b) induced by the intra-arterial injection of 1 and 100 ng Paf (left) and 10 µg ovalbumin (Ovalb, right) to isolate perfused lungs from actively sensitized guinea-pigs in the absence (A) and in the presence of 10 µM WEB 2086 (B), 0.1 mM BN 52021 (C) and 10 µM CV 6209 (D). Fractions of lung effluent were collected before, during the first 3 min and between the 3rd and 6th min after successive injections of Paf and ovalbumin, as described in the Methods section. Amounts of immunoreactive LTC₄-like material and histamine are expressed as ng ml⁻¹ of the values obtained at the peak release, i.e. between 3 and 6 min for LTC₄-like material and between 1 and 3 min for histamine. Results are expressed as mean of 5 to 8 experiments; s.e.mean shown by vertical bars.

Figure 5 Release of histamine (a) and immunoreactive leukotriene C₄ (LTC₄)-like material (b) induced by the intra-arterial injection of 1 and 100 ng Paf (left) and 10 µg ovalbumin (Ovalb, right) to isolate perfused lungs from actively sensitized guinea-pigs in the absence (open columns) and in the presence of Ro 19-3704 (solid columns) and Ro 19-1400 (lined columns), at the concentration of 10 µM. The experimental protocol was the same as the one described in the legend of Figure 4. Results are expressed as mean of 5 to 8 experiments; vertical bars show s.e.mean. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

were measured in the presence and in the absence of the two Paf antagonists.

Interference of the antagonists with Paf- and antigen-induced immunoreactive LTC₄-like material and histamine release from perfused guinea-pig lungs

CV 6209 (Figure 2). By contrast, when the lungs from actively sensitized guinea-pigs were perfused with the Krebs-BSA solution supplemented with Ro 19-3704 or Ro 19-1400 (10 µM), a marked inhibition of bronchoconstriction induced by 1 ng Paf was observed (Figure 1). Ro 19-3704 and Ro 19-1400 also strongly antagonized tracheal strip contractions following the administration of 1 ng Paf and significantly reduced that evoked by antigen challenge (Figure 3).

The possibility that Ro 19-3704 and Ro 19-1400 might affect the reactivity of the tracheal tissue to exogenous stimuli was ruled out, since 940 ± 110 and 965 ± 155 mg contractions to 2 pmol U 46619

Intra-arterial administration of 1 ng Paf into lungs from actively sensitized guinea-pigs evoked the release of much less histamine and immunoreactive LTC₄-like material in the effluent than did antigen (Figure 4). Histamine release was maximal during the first 3 min after the injection of Paf, whereas the peak of immunoreactive LTC₄-like material production was reached between the 3rd and 6th min. Upon injection of 100 ng of the phospholipid, lower amounts of both mediators were detected in the effluent (Figure 4). When ovalbumin was injected last, the amounts of immunoreactive LTC₄-like

material and histamine measured in the effluent were approximately 10 fold above those released upon administration of 1 ng Paf (Figure 4). In the presence of 10 μ M WEB 2086 or CV 6209 or 0.1 mM BN 52021 the release of histamine and of LTC₄-like material following Paf or ovalbumin administration was unaffected (Figure 4).

When the experiments were performed in the presence of Ro 19-3704 and Ro 19-1400 at 10 μ M, release of LTC₄-like material and histamine secretion evoked by the intra-arterial administration of 1 and 100 ng Paf into lungs from actively sensitized guinea-pigs was markedly reduced (Figure 5). Following the administration of ovalbumin the release of LTC₄-like material and histamine was also inhibited by both antagonists (Figure 5).

In these experiments and in those illustrated in Figure 6, the background values for mediator release measured prior to challenge were: histamine, 1.20 ± 0.30 ng ml⁻¹ and LTC₄ 0.68 ± 0.20 ng ml⁻¹.

Interference of Ro 19-3704 and Ro 19-1400 with arachidonic acid-induced LTC₄-like material and histamine release from lungs from actively sensitized guinea-pigs

LTC₄-like material and histamine were released following the injection of 10 μ g arachidonic acid into lungs from actively sensitized guinea-pigs (Figure 6). In the presence of 10 μ M Ro 19-3704 or 19-1400, a marked inhibition of LTC₄-like material production was observed, whereas the release of histamine was only partially reduced (Figure 6).

Kinetics of bronchopulmonary response and mediator release: effect of WEB 2086

To determine the possible relationship between the development of lung hyperresponsiveness and the lack of effect of Paf antagonists, the interference of WEB 2086 with the bronchopulmonary and secretory response to Paf of lungs from guinea-pigs killed at various time intervals during the sensitization period was investigated. As shown in Figure 7, at days 0 and 14, bronchoconstriction induced by 1 ng Paf was minimal as compared to the situation prevailing later and was inhibited by WEB 2086. At this time, neither histamine was released nor was the trachea contracted. By contrast, 4 days after the booster injection of the antigen performed at day 14, lung hyper-responsiveness to Paf developed, reached its maximum at day 21 and persisted for at least 3 months. Concomitantly, in lungs obtained 7 days after the booster injection and up to day 98, bronchoconstriction and mediator release in response to 1 ng Paf were unaffected by WEB 2086 (Figure 7).

Figure 6 Release of histamine (a) (upper panel) and immunoreactive leukotriene C₄ (LTC₄)-like material (b) induced by the intra-arterial injection of 10 μ g arachidonic acid to isolated perfused lungs from actively sensitized guinea-pigs in the absence (open columns) and in the presence of Ro 19-3704 (solid columns) and Ro 19-1400 (lined columns), both used at the concentration of 10 μ M. The experimental protocol was the same as the one described in the legend of Figure 4. Results are expressed as mean of 4 experiments; s.e.mean shown by vertical bars. * $P < 0.05$; *** $P < 0.001$.

Similar results were obtained upon injection of lower doses (0.1 and 0.01 ng) of Paf (Table 1). Interestingly, when the booster injection was omitted and the experiments were performed at day 21, the lung response to Paf was similar to that observed at day 0 and 14 and bronchoconstriction was suppressed by WEB 2086 (Figure 7).

Table 1 Effect of WEB 2086 (10 μ M) on Paf (0.1 and 0.01 ng)-induced bronchoconstriction (BC) and mediator release from isolated perfused lungs from actively sensitized guinea-pigs

Paf (ng)	BC (%)	Histamine (ng ml ⁻¹)	Trachea tension (mg)
0.1	100	19.0 $\pm$ 4.9	1120.0 $\pm$ 381.6
0.1 (+ WEB 2086)	100	11.8 $\pm$ 0.7	720.0 $\pm$ 69.3
0.01	100	26.8 $\pm$ 6.0	693.3 $\pm$ 70.6
0.01 (+ WEB 2086)	100	32.3 $\pm$ 6.4	740.0 $\pm$ 207.3

Results are expressed as mean $\pm$ s.e.mean of 3–6 experiments.

Figure 7 Bronchoconstriction (a), tracheal contraction (b) and histamine release (c) induced by the intra-arterial administration of 1 ng Paf into lungs from actively sensitized guinea-pigs in the absence (open columns) and in the presence of 10 μ M WEB 2086 (stippled columns). The animals were killed at defined time intervals after the first sensitizing injection at day 0. The booster injection of the antigen was performed at day 14. Bronchoconstriction is expressed as % increase of the bronchial resistance to inflation measured prior to the administration of Paf. Tracheal strip response is expressed in mg tension. Results are expressed as mean of 5 to 7 experiments; s.e.mean shown by vertical bars. * $P < 0.05$; *** $P < 0.001$.

Effect of Paf antagonists on Paf- and antigen-induced lung oedema formation

When lungs from actively sensitized guinea-pigs were injected at 10 min intervals with 0.1 ml saline containing 0.1% BSA, only a slight increase ($35.5 \pm 6.6\%$, $n = 5$) of the wet weight was observed after 40 min (Figure 8). By contrast, following the injection of 1 and 100 ng Paf, the lung weight was markedly increased (Figure 8). A further increase was

Figure 8 Interference of the various Paf antagonists with lung oedema formation, expressed by the % increase in lung wet weight. In (a) are shown the increments in lung weight measured in lungs perfused for 40 min and injected at 10 min intervals with 0.1 ml saline containing 0.1% BSA (column A); after the injection of 1 and 100 ng Paf (column B) and at the end of the experiments, after ovalbumin administration (column C). In (b), the increase of the lung wet weight measured at the end of the experiments (column C) is compared to that obtained in the presence of 10 μ M WEB 2086 (column D), 0.1 mM BN 52021 (column E), 10 μ M CV 6209 (column F), 10 μ M Ro 19-3704 (column G) and 10 μ M Ro 19-1400 (column H). Data are expressed as mean of 5 to 8 experiments; s.e.mean shown by vertical bars. *** $P < 0.001$.

measured at the end of the experiment, after ovalbumin administration (Figure 8). In the presence of the Paf antagonists, the increase of lung wet weight due to either Paf or ovalbumin was suppressed (Figure 8).

Discussion

The present results confirm our claim that the responsiveness to Paf of lungs of actively sensitized guinea-pigs is markedly increased when compared to that of lungs of non-immunized animals and that the pharmacological modulation of the bronchopulmonary and secretory effects of Paf in sensitized lungs is different from that of control preparations (Pretolani *et al.*, 1988). Indeed, bronchoconstriction to Paf in lungs from actively sensitized animals is not mediated by products from the cyclo-oxygenase

pathway (Pretolani *et al.*, 1988), whereas cyclo-oxygenase inhibitors are effective against Paf-induced bronchoconstriction in non-sensitized lungs (Lefort *et al.*, 1984). This observation was further strengthened by the fact that lipoxygenase metabolites of arachidonic acid play a major role in Paf-induced bronchoconstriction in lungs from actively sensitized guinea-pigs (Pretolani *et al.*, 1989).

In the present work we demonstrate that the potent Paf antagonists WEB 2086, BN 52021 and CV 6209 become ineffective against Paf-induced bronchoconstriction and release of leukotriene-like materials and histamine when the mediator is administered to lungs from actively sensitized guinea-pigs. This phenomenon does not result simply from a shift in the potency of the drugs since the experiments were performed with concentrations of Paf antagonists 10 to 100 fold above those effective in control preparations. Furthermore, in the time-course experiments, we observed that failure of WEB 2086 was strictly related to the development of the lung hyper-responsiveness which follows the booster injection of the antigen. In confirmation, the response to Paf of lungs from animals which did not receive the booster injection at day 14 was similar to that of control preparations and was suppressed by WEB 2086. This supports our previous contention (Pretolani *et al.*, 1988) that the hyper-responsiveness to Paf of lungs from actively sensitized guinea-pigs is triggered by the booster injection of the antigen which leads to a marked modification of the lung reactivity to the phospholipid mediator.

Even though WEB 2086, BN 52021 and CV 6209 were ineffective against the bronchopulmonary effects of Paf in sensitized lungs, the other two antagonists Ro 19-3704 and Ro 19-1400 retained their activity against Paf. However, inhibition by Ro 19-3704 and Ro 19-1400 of the release of immunoreactive LTC₄-like material induced by arachidonic acid indicates that these compounds are not specific Paf antagonists and that they may interfere with the activation of the lipoxygenase pathway. This concept is reinforced by the ability of Ro 19-3074 and Ro 19-1400 to inhibit the release of LTC₄-like material induced by the antigen administration. The blockade of the generation of lipoxygenase metabolites by both compounds strongly supports a role of leukotrienes in the bronchopulmonary manifestations induced by Paf in sensitized lungs and agrees with our demonstration of the ability of the mixed cyclo-oxygenase and lipoxygenase inhibitor BW 755C (but not of pure cyclo-oxygenase inhibitors) to counteract bronchoconstriction by Paf (Pretolani *et al.*, 1989). The interference by Ro 19-3704 and Ro 19-1400 with the release of peptido-leukotrienes evoked by agonists other than Paf also agrees with data of Lagente *et al.* (1988) showing that Ro 19-3704 inhibits the

production of superoxide anions from guinea-pig alveolar macrophages stimulated with various agonists, including the secretagogue tripeptide FMLP. Furthermore, recent results from our laboratory (Desquand *et al.*, 1988) show that Ro 19-3704 impairs the release of thromboxane B₂ and LTB₄ and the production of superoxide anions following stimulation of rabbit polymorphonuclear neutrophils with opsonized-zymosan and of guinea-pig alveolar macrophages with FMLP.

Platelet activating factor has the ability to increase pulmonary vascular permeability *in vivo* and *in vitro* (Bureau *et al.*, 1987; Evans *et al.*, 1987a; Imai *et al.*, 1988) and, accordingly, its specific antagonists inhibit this phenomenon (Evans *et al.*, 1987a; Imai *et al.*, 1988). In our present experiments, the Paf antagonists which failed to inhibit bronchoconstriction and release of mediators from lungs from actively sensitized guinea-pigs by Paf itself suppressed altogether Paf- and antigen-induced oedema formation. This indicates that the recognized Paf antagonists continue to block the effects of the agonist on resident endothelial cells, which account for permeability changes. We recently showed that BW 755C is as effective as Paf antagonists in inhibiting the increase in lung weight due to Paf and antigen (Pretolani *et al.*, 1989). Since those experiments were performed in the presence of indomethacin, it is likely that only the products of the lipoxygenase pathway account for the development of oedema induced by Paf in the perfused guinea-pig lung. Indeed, leukotrienes have been also demonstrated to induce alterations in lung vascular permeability (Evans *et al.*, 1987b). Thus, it can be hypothesized that following the immunological stimulations, several mediators are released including Paf, which then triggers the production of leukotrienes creating a mediator network, as already suggested by Murphy & Henson (1985).

It is possible that the effects of Paf which become refractory to the antagonists that were used are mediated through another receptor, either brought in by hypothesized inflammatory cells that it is suggested migrate to the lungs in response to the booster injection of the antigen or modified upon the resident cells, particularly mast cells responsible for histamine release. This receptor might not be recognized by the antagonists employed. Nevertheless, the efficacy of Ro 19-3704 and Ro 19-1400, under conditions where CV 6209 was inactive, does not support this possibility, since the three agents are Paf structural analogues. Alternatively, the effects of Paf may become non-receptor mediated, even though this concept is unclear presently.

In conclusion, our results demonstrate that: (i) WEB 2086, BN 52021 and CV 6209 fail to inhibit bronchoconstriction and mediator release induced by Paf administered to lungs from sensitized guinea-

pigs; (ii) the production of Paf by the lungs during anaphylaxis (Fitzgerald *et al.*, 1986) is important for the development of oedema, since the antagonists tested prevented the increase in lung wet weight following ovalbumin administration; (iii) the activation of the lipoxygenase pathway is involved in the increased reactivity of lungs from boosted animals.

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The authors wish to thank Prof B. David and Ms M. Laurent (Unité d'Immunoallergie, Institut Pasteur) for histamine determinations.

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(Received October 11, 1988

Revised January 10, 1989

Accepted February 2, 1989)