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Pharmacological modulation of the effects of *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine in guinea-pigs: involvement of the arachidonic acid cascade

M. Abdelhakim Boukili, Michel Bureau, Vincent Lagente, Jean Lefort, Arielle Lellouch-Tubiana*, Evelyne Malanchère & B. Boris Vargaftig

Unité Associée Institut Pasteur/INSERM no. 285, Departement de Physiopathologie Expérimentale, Institut Pasteur, 28 rue du Dr Roux, Paris 75015 and Departement d'Histologie-Embryologie*, Faculté de Médecine Necker, 156 Rue de Vaugirard, Paris 75015, France

1 The intravenous administration of the chemotactic and secretagogue peptide *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP; 0.3–30 $\mu g\,kg^{-1}$) to the guinea-pig induces bronchoconstriction and dose-dependent leukopenia accompanied by mild thrombocytopenia. No electron microscopic evidence of platelet aggregation in lungs or significant accumulation of ^{111}In-labelled platelets in the thoracic region at the height of bronchoconstriction was noted.

2 Bronchoconstriction and leukopenia induced by FMLP were not affected by prostacyclin, by platelet depletion, by the platelet-activating factor (Paf-acether) antagonist BN 52021 or by the histamine H_1-antagonist mepyramine. Bronchoconstriction, but not leukopenia, was inhibited by aspirin, whereas the peptido-leukotriene antagonist compound FPL 55712 and the cyclo-oxygenase lipoxygenase inhibitor indomethacin reduced bronchoconstriction to a limited extent only. The mixed cyclo-oxygenase/lipoxygenase inhibitor compound BW 755C was very effective in blocking bronchoconstriction by the highest dose of FMLP used, but failed to interfere with leukopenia.

3 FMLP-induced dose-dependent contraction of parenchymal lung strips was accompanied by the formation of immuno-reactive thromboxane B_2 in amounts markedly less than those formed from exogenous arachidonic acid at concentrations equieffective in inducing contractions.

4 FMLP-induced contractions of the guinea-pig lung strip were not modified by mepyramine nor by FPL 55712. They were reduced by indomethacin and aspirin and an even greater reduction was obtained with aspirin used in combination with FPL 55712. BW 755C suppressed the effects of all the concentrations of FMLP tested, whereas tert-butyloxy-carbonyl-L-methionyl-L-leucyl-L-phenylalanine, a chemical analogue of FMLP, displaced the concentration-response curve to the right, without reducing the maximal contraction obtained.

5 The present results indicate that: (a) bronchoconstriction by FMLP is not due to platelet activation, to cyclo-oxygenase-dependent mechanisms or to peptido-leukotriene formation. The inhibitory effect of aspirin and BW 755C involves a property other than cyclo-oxygenase inhibition, which is not shared by indomethacin. (b) The contractile effects of FMLP on parenchymal lung strips follow an interaction with specific receptor sites, as shown by the effectiveness of tert-butyloxy-carbonyl-L-methionyl-L-leucyl-L-phenylalanine, and involves the combined effects of cyclo-oxygenase and lipoxygenase metabolites.

Introduction

Guinea-pigs undergo bronchoconstriction and thrombocytopenia after systemic chemical or immune stimuli, because of the release and/or the formation of a variety of mediators (Samuelsson, 1983). Thus, the synthesis of thromboxane A_2 (TXA_2) accounts for the bronchoconstriction and thrombocytopenia seen after an intravenous injection of arachidonic acid (Lefort & Vargaftig, 1978; Vargaftig & Lefort, 1979). TXA_2

formation is not restricted to platelets since it also occurs in blood-free perfused lungs (Vargaftig & Dao, 1971). Accordingly, platelet-depleted or prostacyclin-treated animals still respond with bronchoconstriction following an intravenous (i.v.) injection of arachidonic acid (reviewed in Vargaftig *et al.*, 1981b). In contrast, bronchoconstriction following i.v. collagen (Vargaftig *et al.*, 1979), ADP and ATP (Lefort & Vargaftig, 1978) and platelet-activating factor (Paf-acether) (Vargaftig *et al.*, 1980) is platelet-dependent and is suppressed when platelets are depleted or thrombocytopenia is inhibited with prostacyclin. In this case, sequestration of platelet aggregates is not the major determinant of platelet-induced bronchoconstriction, which involves the release of mediators from trapped platelets at the vicinity of the respiratory smooth muscle (Page *et al.*, 1982; Lellouch-Tubiana *et al.*, 1985; Pretolani *et al.*, 1986).

The complex relationship between aggregation and vascular trapping of platelets and bronchoconstriction are thus being clarified. In contrast, the relationship between polymorphonuclear leukocyte (PMNL) activation and bronchoconstriction are less well documented. This may be particularly important because of the role of PMNLs in pulmonary inflammation (Staub *et al.*, 1982) and in view of the possibility that neutrophils and/or eosinophils participate in late asthma (Cohen *et al.*, 1981; Nagy *et al.*, 1982; Durham *et al.*, 1984). The interaction between PMNL and bronchoconstriction has been studied using *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP), which lacks direct platelet-stimulating effects, but is a potent PMNL secretagogue and induces inflammatory responses, accompanied by PMNL accumulation when injected into animals (Hamel *et al.*,1984; Pham Huy *et al.*, 1985; Olsen *et al.*, 1986) and man (E. Henocq, personal communication). In addition, the direct effects of FMLP on isolated lung strips were studied and potential inhibitors were tested.

Methods

Measurement of bronchoconstriction

Hartley guinea-pigs of either sex (250–500 g) were anaesthetized with pentobarbitone (40 mg kg^{-1} i.v.), spontaneous breathing was suppressed with pancuronium (2 mg) and propranolol was administered (1 mg kg^{-1} i.v. and 3 mg kg^{-1} i.p). One jugular vein was cannulated for injection of drugs. Both carotid arteries were catheterized, one to monitor arterial blood pressure and the other for blood sampling (see below). Bronchial reactivity was tested with 5-HT (1–2 $\mu g\,kg^{-1}$) at 10 min intervals until three identical consecutive responses (25–30 cmH_2O) were obtained. The animals were given FMLP, 0.3, 1, 3, 10 and 30 $\mu g\,kg^{-1}$ i.v., at 45 min intervals. Bronchoconstriction, evaluated according to the pulmonary resistance to inflation, and arterial blood pressure were recorded on a Beckman Dynograph R 511.

Measurement of platelet sequestration in the lung

Autologous platelets were radiolabelled with 20 μCi [^{111}In]-oxinate as described elsewhere (Page *et al.*, 1982) and then injected intravenously (1 ml) one hour before FMLP administration. ^{111}In radioactivity in the lung was counted externally with a collimated detector (Scintillation counter DM1-1; Nuclear Enterprises Edinburgh, U.K.) connected to a Pulse height analyser (PHA 1; Numelec, France).

Blood cell counts

Blood (200 μl) was collected from the carotid artery and the total leukocyte and platelet concentration was estimated with a Coulter Counter ZBI before, 10 and 60 s after FMLP administration.

Electron microscopy studies

FMLP, 3 $\mu g\,kg^{-1}$, was administered into the cannulated jugular vein and lungs were removed one min later. Small fragments were minced into slices and immediately immersed in cold 2.5% glutaraldehyde for 24 h. The specimens were then rinsed in phosphate buffer and post-fixed in 1% osmic acid, dehydrated and embedded in Epon (Luft, 1961). One micrometer sections stained with toluidine blue were used to select areas from which thin sections were prepared and stained with uranyl acetate and lead citrate. Sections were then examined in a Philips EM 300 transmission electron microscope.

Studies on parenchymal lung strips

Six peripheral strips were dissected from lungs, thoroughly washed via the pulmonary artery with 50 ml of Krebs solution, and mounted in 16 ml organ baths containing the same solution, which was continuously aerated with a mixture of 95% O_2 and 5% CO_2 and maintained at 37°C. The composition of the Krebs solution was (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, glucose 5.6. One end of each strip was connected to a Statham force displacement transducer under a resting tension of 2 g. One hour was allowed for equilibration and acetylcholine was then added cumulatively (0.1, 1 and 10 μM) at 90 s intervals. After three washings and 15 min later, FMLP was added, at final concentrations of 1 and 3 nM, at 20 min intervals. Then, after a further interval of 20 min, 10, 30, 100 and

300 nM FMLP was added in the absence or presence of the different potential antagonists, which were left in contact with the tissues for 4 min.

Radioimmunoassay of thromboxane B_2

Thromboxane B_2 was assayed by the technique of Sors *et al.* (1978). Aliquots (100 µl) collected from the organ bath before and 1, 3 and 10 min after each addition of FMLP were left at room temperature for 60 min. For performing the radioimmunoassay, the samples were thawed and incubated overnight at 4°C with iodine-labelled TXB_2 and anti-TXB_2 serum in a gamma-globulin buffer. The next day, bound and free fractions were separated by polyethyleneglycol 6000 precipitation followed by centrifugation at 2000 *g* at 4°C for 10 min. The radioactivity of the pellet corresponding to the bound fraction was counted for 1 min with an 'Intertechnique' gamma counter directly connected to a Hewlett-Packard calculator.

Statistical analysis

Statistical significance was evaluated by Student's *t* test. All data are expressed as mean ± s.e. mean.

Drugs used

Sodium pentobarbitone (Clin Midy, France), pancuronium (Pavulon, Organon, France), propranolol (ICI, U.K.), indomethacin, prostacyclin, 5-hydroxytryptamine (5-HT), acetylcholine, arachidonic acid, salicylic acid, bovine gammaglobulin, *N*-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP), *N*-tert-butyloxy-carbonyl-L-methionyl-L-leucyl-L-phenylalanin, (Sigma, U.S.A.); aspirin as lysine acetylsalicylate (Aspegic, Laboratoires Egic, Paris, France), sodium 7-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxy propoxy)-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylate (FPL 55712; Fisons, Loughborough, U.K.), 3-amino-1-(m-(trifluoromethyl) phenyl)-2-pyrazoline (BW 755C; The Wellcome Research Laboratories, Beckenham, U.K.), 9H-1, 7a-(epoxymethano)-1H, 6 a H-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,-7b,-11 hydroxy-8 methyl (BN 52021; Institut Henri Beaufour, Le Plessis Robinson, France), 1-*O*-alkyl-2-acetyl-sn-glycero- 3-phosphocholine (Paf-acether, gift by Prof. J.J. Godfroid, Paris VII University, France), mepyramine maleate (Rhone-Poulenc, France), [^{111}In]-oxinate (Amersham, U.K.), absolute ethanol, sodium carbonate ($Na_2CO_3 10H_2O$), polyethyleneglycol 6000 (Merck, Munchen, R.F.A.), radiolabelled TXB_2 and anti-TXB_2 serum (Institut Pasteur, Paris). Anti-platelet serum was prepared as described previously (Lefort & Vargaftig, 1978).

Preparation of indomethacin Indomethacin was solubilized either in 1 N NaOH and then adjusted to pH 7.8–8.0, or dissolved in absolute ethanol (100 mg ml^{-1}), and further diluted with sodium carbonate (0.1 M).

Results

In vivo *effects of FMLP*

The i.v. administration of FMLP was followed by a dose-dependent bronchoconstriction and by a marked reduction of the number of circulating PMNL, accompanied by a moderate thrombocytopenia (Figures 1a and 2). Smears were obtained in three cases from blood collected before and after the administration of 3 µg kg^{-1} FMLP. Neutrophils represented 64% of the total PMNL (5500 ± 1650), and dropped to 27% and 8%, 10 and 60 s respectively, after FMLP.

Electron microscopy studies

Animals were killed 1 min after an i.v. injection of 3 µg kg^{-1} FMLP, and the lungs were removed as described in Methods. Neutrophil sequestration developed in the pulmonary vascular venules, arterioles and capillaries. These cells were commonly associated with monocytes (Figure 3a and b). In contrast, platelet accumulation in the vessels was never observed. Neutrophils accompanied by monocytes, and occasionally by eosinophils, were also present in the septa and in the connective tissue surrounding the bronchioles and arteries. Some of these inflammatory cells were partially or completely degranulated (Figure 3b). Focal accumulation of inflammatory cells was also noted in the alveolar spaces, which contained numerous activated macrophages with fused membranes (Figure 3c). The electron microscopic findings were not modified when the animal was pretreated with 20 mg kg^{-1} aspirin, a dose which suppressed bronchoconstriction (see below). In contrast to aspirin, no neutrophil accumulation was observed in indomethacin-treated animals (Figure 3d), even though alveolar macrophages were as activated as in control FMLP-treated animals.

Pharmacological modulation of the effects of FMLP

Bronchoconstriction induced by Paf-acether (Vargaftig *et al.*, 1980) or by collagen (Vargaftig & Lefort, 1979; Vargaftig *et al.*, 1979) is suppressed by immune platelet depletion. To verify if bronchoconstriction by FMLP is platelet-dependent as well, three guinea-pigs were infused with 1 ml kg^{-1} anti-platelet serum, over a period of 30 min. This reduced the platelet counts from $390 \pm 22 \times 10^3$ mm^{-3} to $< 10 \times 10^3$ mm^{-3}. Under

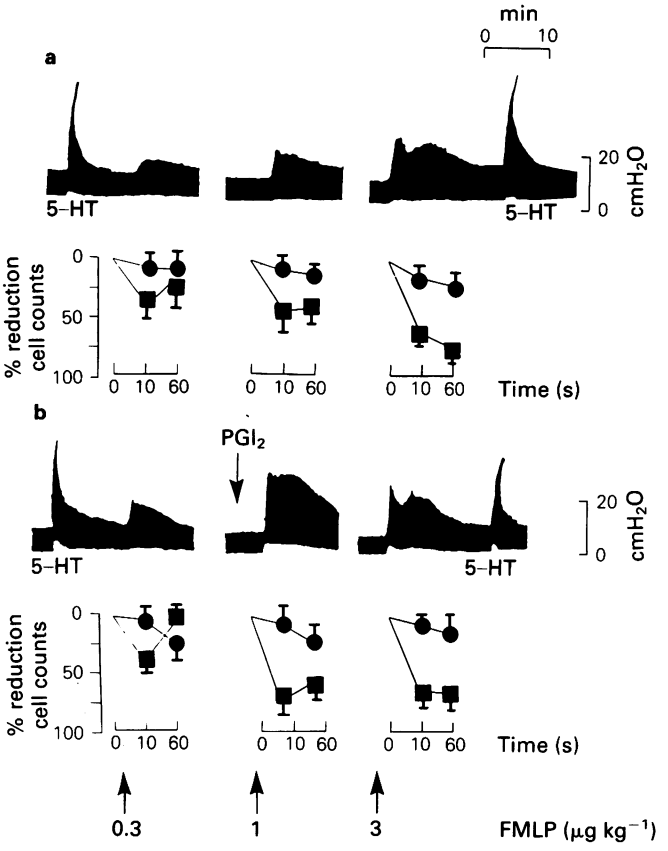

Figure 1 Typical tracings of the bronchoconstriction produced by *N*-formyl methionyl leucyl phenylalanine (FMLP, upper panels of (a) and (b)) and percentage reduction of the platelet (●) and polymorphonuclear leukocyte (■) counts (lower panels of (a) and (b)) 10 and 60 s after i.v. injections of 0.3, 1 and 3 $\mu g\,kg^{-1}$ FMLP (n = 12). In (b) (arrow), 1 $\mu g\,kg^{-1}$ prostacyclin (PGI_2) was perfused i.v. 1 min immediately before 1 $\mu g\,kg^{-1}$ FMLP. 5-Hydroxytryptamine (5-HT, 1 $\mu g\,kg^{-1}$) was used to evaluate the responsiveness of the airways. Vertical lines indicate s.e.mean.

those conditions, neither the bronchoconstriction nor the leukopenia induced by FMLP were modified (Figure 4). Similarly, the platelet protective agent prostacyclin given at a dose of 1 $\mu g\,kg^{-1}$ for 1 min failed to block the bronchoconstriction and appeared even to augment it slightly. The thrombocytopenic and leukopenic effects of FMLP were also unaffected by prostacyclin (Figure 1b).

BN 52021 which at 0.1–0.3 $mg\,kg^{-1}$ suppresses the bronchoconstrictor effects of Paf-acether (Desquand *et al.*, 1986) was also inactive against FMLP. Thus the bronchoconstriction induced by 3 $\mu g\,kg^{-1}$ FMLP was 12 ± 2 cmH_2O before and 20 ± 4.6 $cm\,H_2O$ after 1 $mg\,kg^{-1}$ BN 52021 ($n = 3$; no statistical difference).

No platelet sequestration was seen following the injection of FMLP (1 and 3 $\mu g\,kg^{-1}$) to animals containing radiolabelled platelets, under conditions where an i.v. injection of 33 $ng\,kg^{-1}$ Paf-acether to the same animal induced a significant platelet accumulation in the lung, reaching a maximum increase in ^{111}In content of $20 \pm 3\%$ above the basal level (Figure 5, representative of 3 similar experiments).

Aspirin inhibits arachidonic acid-induced bronchoconstriction, thrombocytopenia (Lefort & Vargaftig, 1978) and leukopenia (our unpublished data) in the guinea-pig. As seen in Figure 6, aspirin also suppressed the bronchoconstriction induced by 3 $\mu g\,kg^{-1}$ FMLP, and inhibited most of the effects of 10–30 $\mu g\,kg^{-1}$ FMLP. Salicylic acid was inactive; the bronchoconstriction induced by 3 $\mu g\,kg^{-1}$ FMLP was

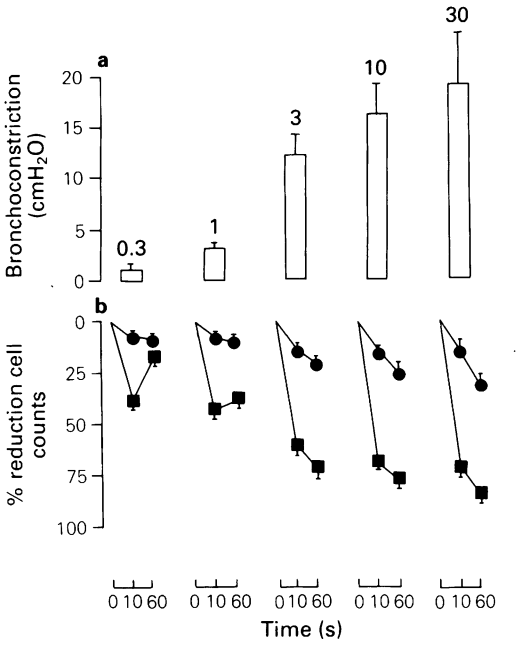

Figure 2 Dose-dependent activity of *N*-formyl methionyl leucyl phenylalanine (FMLP). (a) Shows the intensity of bronchoconstriction induced by FMLP 0.3, 1, 3, 10 and 30 $\mu g\,kg^{-1}$. (b) Shows the percentage reduction of platelet (●) and polymorphonuclear leukocyte (■) counts 10 and 60 s after the i.v. injections of FMLP. Vertical lines indicate s.e.mean. $n = 12$.

12 cmH_2O before and of 13.9 cmH_2O after 20 $mg\,kg^{-1}$ salicylic acid ($n = 2$). The thrombocytopenic and leukopenic effects of 3 $\mu g\,kg^{-1}$ FMLP were not modified by aspirin. Thus 1 min after 3 $\mu g\,kg^{-1}$ FMLP the leucocyte blood concentration dropped by $73 \pm 4\%$, as compared to $75 \pm 3\%$ 10 min after 20 $mg\,kg^{-1}$ aspirin. Similarly, the platelet concentration was reduced by $21 \pm 4\%$ by 3 $\mu g\,kg^{-1}$ FMLP before, and by $16 \pm 2\%$ after, aspirin. The leukotriene antagonist FPL 55712 (10 $mg\,kg^{-1}$, 30 s before FMLP) failed to interfere with the effects of FMLP (Figures 4 and 6). The anti-histamine mepyramine (0.2 $mg\,kg^{-1}$) was also inactive since the bronchoconstriction induced by 3 $\mu g\,kg^{-1}$ FMLP injected to control animals was $12 \pm 2\,cmH_2O$ as compared to $13 \pm 0.5\,cmH_2O$ in mepyramine-treated animals ($n = 4$). Furthermore, indomethacin did not cause a significant reduction in the bronchoconstriction induced by 3 and 10 $\mu g\,kg^{-1}$ FMLP (Figure 6). Since the effectiveness of aspirin contrasted with the relative ineffectiveness of indomethacin, both being cyclo-oxygenase inhibitors, indomethacin was solubilized under two different conditions (see Methods), with essentially the same negative results. In concentrations up to 10 $mg\,kg^{-1}$, indomethacin also failed to interfere with the leukopenia induced by FMLP. Both solutions of indomethacin used were effective against arachidonic acid-induced platelet aggregation, indicating that the indomethacin had not lost its activity.

The mixed cyclo-oxygenase lipoxygenase inhibitor, BW 755C (10 $mg\,kg^{-1}$) inhibited bronchoconstriction induced by FMLP 3 $\mu g\,kg^{-1}$ ($78 \pm 12\%$ reduction, $n = 3$) and 30 $\mu g\,kg^{-1}$ (Figure 7) but did not interfere with leukopenia. At the same dose, BW 755C suppressed arachidonic acid-induced bronchoconstriction and thrombocytopenia (not shown). One hour after the administration of BW 755C, the bronchoconstriction induced by arachidonic acid and FMLP was again obtained (Figure 7).

Contraction by FMLP of the isolated parenchymal lung strip and stimulation of thromboxane B_2 synthesis

FMLP induced a concentration-dependent contraction of the lung strips accompanied by synthesis of TXB_2 (Figure 8). These contractions were the same regardless of whether the lungs were initially washed intra-arterially with 50 ml of Krebs solution or inhibited by the anti-histamine mepyramine (1 μM). Indeed, the contractions of the lung strips triggered by 10 and 30 nM FMLP were 91 ± 16 and 170 ± 19.5 mg, respectively, in the presence of mepyramine, and were 121 ± 26 and 191 ± 29 mg, respectively, in its absence. Tert-butyloxy-carbonyl-L-methionyl-L-leucyl-L-phenylalanine, a chemical analogue of FMLP described as a competitive inhibitor (Marone *et al.*, 1984) displaced the concentration-dependent curve for FMLP to the right, without reducing significantly the maximal contractile effect (Figure 9). Arachidonic acid (1, 10, 100 and 1000 μM) also induced concentration-dependent contractions of the lung strips accompanied by the biosynthesis of TXB_2, which matched the amounts obtained with 3, 30, 300 and 3000 nM FMLP (Figure 8). The contractile effect of 1 mM arachidonic acid was less marked, even though the synthesis of TXB_2 persisted. The total amounts of TXB_2 formed 10 min after the addition of FMLP to the organ bath were approximately 6 times lower than those obtained with 1 mM arachidonic acid. Aspirin (0.1 mM) suppressed up to 95% of this formation (not shown), whether triggered by arachidonic acid or FMLP, but only blocked 40–60% of the contractile effect of FMLP, at all final concentrations used (Figure 10). Indomethacin (1 μM) (Figure 11) also reduced the contractile effects of FMLP, but the inhibition was surmounted by higher concentrations of FMLP. This residual contractile activity might be accounted for by non-cyclo-oxygenase mediators, such as the leukotrienes. Nevertheless, the peptido-leukotriene antagonist FPL 55712 (Figure 12), only reduced the effects of 10

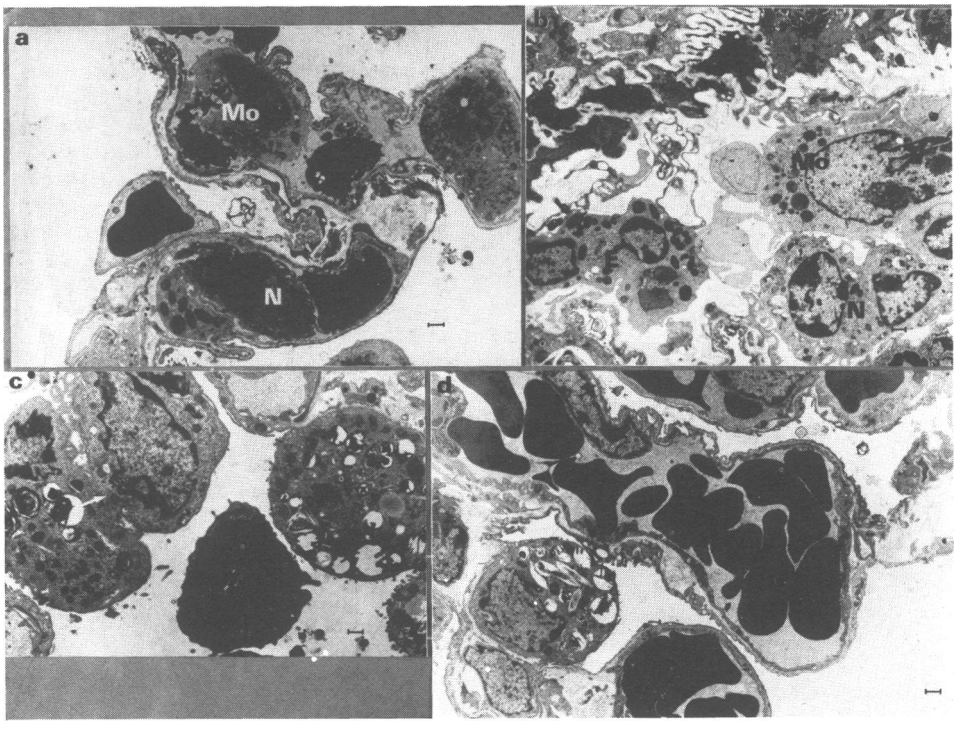

Figure 3 Electron micrographs obtained one minuite after an intravenous injection of 3 $\mu g\ kg^{-1}$ *N*-formyl methionyl leucyl phenylalanine (FMLP). (a) Part of an interalveolar septum; note the accumulation in the capillary lumen of neutrophils (N) associated to monocytes (Mo), scale bar = 1 μm. (b) A lung arteriole showing a contracted internal elastic lamina. Degranulated neutrophils (N) are seen associated with monocytes (Mo) and eosinophils (E), scale bar = 1 μm. (c) The alveolar lumen associated with an eosinophil and macrophages fusion is observed (see arrow), scale bar = 1 μm. (d) Interalveolar septa in the lung of indomethacin-pretreated animals. No neutrophils accumulate in the capillary lumen, scale bar = 1 μm.

and 30 nM FMLP and was ineffective against 100 and 300 nM. Even though the inhibitory effects of aspirin and indomethacin were potentiated by FPL 55712 (Figures 10 and 11), a marked contraction still persisted. The only drug which suppressed the contractile response to all concentrations of FMLP was the mixed anti-cyclo-oxygenase/lipoxygenase inhibitor BW 755C (Figure 12).

Discussion

The PMNL secretagogue FMLP induced a dose-dependent bronchoconstriction when injected intravenously to the guinea-pig. Hamel *et al.* (1984) described similar findings but they used doses of FMLP markedly higher than ours, possibly because in their experiments the guinea-pigs were not pretreated with propranolol. Confirming the results of these authors, we now demonstrate that bronchoconstriction is accompanied by dose-dependent leukopenia (neutropenia) and by moderate thrombocytopenia. Neither immune platelet depletion nor i.v. prostacyclin interfered with the bronchopulmonary and leukopenic effects of FMLP (Figures 1b and 4) under conditions where bronchoconstriction by collagen or by Paf-acether is abrogated (Vargaftig *et al.*, 1979; 1980; Vargaftig & Lefort, 1979). In agreement with this conclusion, the electron micrographs showed that neutrophils, eosinophils and monocytes accumulate in the pulmonary arterioles, capillaries and venules one minute after FMLP, but that platelet aggregates were absent. This contrasts with the picture following injections of Paf-acether, when platelet aggregation (Page *et al.*, 1982; 1984) and diapedesis are the major microscopic findings (Lellouch-Tubiana *et al.*, 1985). Moreover, radiolabelled platelets were not retained in the lungs of FMLP-injected animals, under conditions

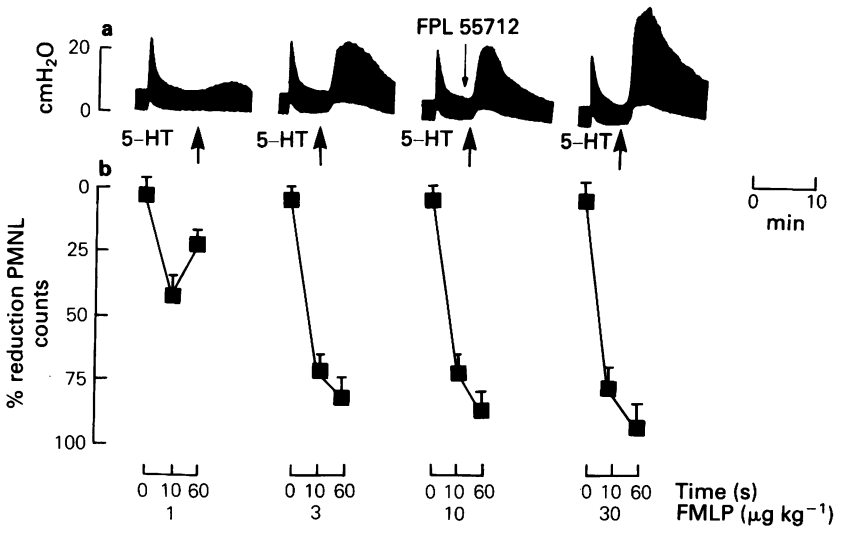

Figure 4 Effect of immune platelet depletion on *N*-formyl methionyl leucyl phenylalanine (FMLP)-induced (a) bronchoconstriction and (b) reduction in polymorphonuclear leukocytes (PMNLs). (a) Bronchoconstriction induced by FMLP after perfusion of anti-platelet serum (1 ml kg^{-1} for 30 min, $n = 3$). FPL 55712 (10 mg kg^{-1}) was injected before FMLP 10 μg kg^{-1}. (b) Percentage reduction of PMNL count before, 10 and 60 s after each i.v. injection of FMLP. 5-HT refers to the injection of 5-hydroxytryptamine (1 μg kg^{-1}). Vertical lines indicate s.e.mean.

where Paf-acether induced such an accumulation (Page *et al.*, 1982). Since the Paf-acether antagonist BN 52021 was also inactive against the bronchoconstriction induced by FMLP, it is clear that i.v. FMLP stimulates circulating PMNL, which are then trapped in the pulmonary vessels, and furthermore that neither platelets nor Paf-acether are involved in FMLP-induced bronchoconstriction.

Indomethacin and aspirin do not block the leukopenia induced by FMLP in the rabbit (Dahinden & Fehr, 1980). We were unable to prevent leukopenia with either drug, but were able to suppress bronchoconstriction with aspirin, and lung PMNL accumulation with indomethacin. This apparent anomaly may be related to the effectiveness of indomethacin against FMLP-induced lysosomal enzyme release (Palmer & Weathrall, 1978). Failure of indomethacin to inhibit bronchoconstriction under

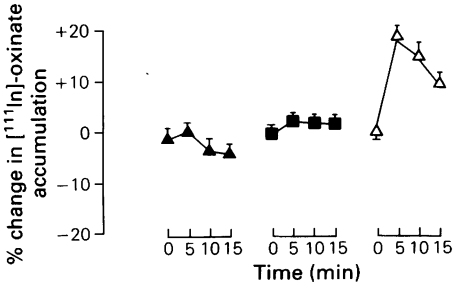

Figure 5 Percentage change of [^{111}In]-oxinate content in the lung before and 5, 10 and 15 min after i.v. injections of *N*-formyl methionyl leucyl phenylalanine (FMLP) 1 (▲) and 3 (■) μg kg^{-1} or platelet-activating factor (Paf-acether) 0.033 μg kg^{-1} (△). Vertical lines indicate s.e.mean.

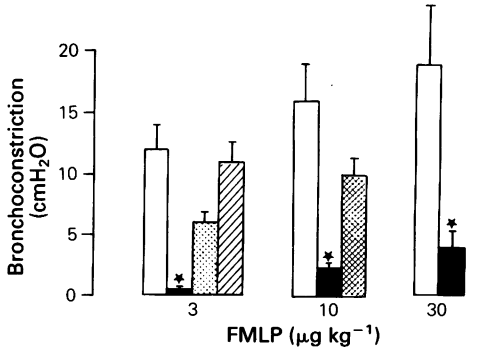

Figure 6 Effect of aspirin (solid columns, 20 mg kg^{-1}, $n = 4$), indomethacin (cross-hatched columns, 10 mg kg^{-1}, $n = 3$) and FPL 55712 (diagonally-hatched column, 10 mg kg^{-1}, $n = 4$) on the bronchoconstriction induced by *N*-formyl methionyl leucyl phenylalanine (FMLP) 3, 10 and/or 30 μg kg^{-1}. Vertical lines indicate s.e.mean. *$P < 0.001$. Open columns represent control responses to FMLP.

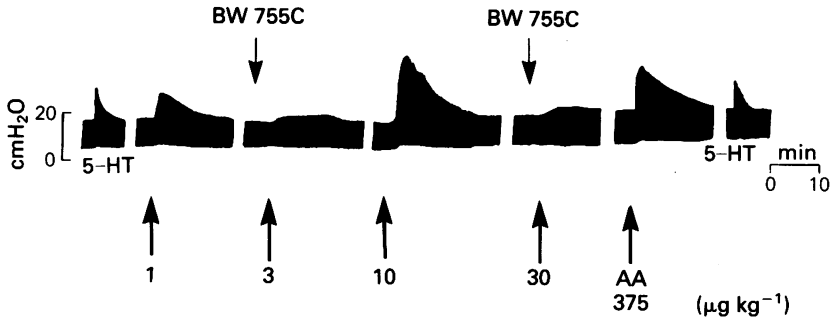

Figure 7 Effect of BW 755C on the bronchoconstriction induced by *N*-formyl methionyl leucyl phenylalanine (FMLP). BW 755C (10 mg kg^{-1}) was injected as indicated 3 min before FMLP (3 and 30 μg kg^{-1}). One hour after its second administration, bronchoconstriction was triggered by arachidonic acid (AA, 375 μg kg^{-1}). 5-HT refers to 5-hydroxytryptamine (1 μg kg^{-1}).

conditions where PMNL accumulation was prevented, argues against PMNLs mediating bronchoconstriction. One possible explanation for this apparent contradiction is that the target for FMLP-induced bronchoconstriction is another cell, for instance the alveolar macrophage, which was found to be activated after i.v. FMLP, and not protected by indomethacin (see Figure 3d). Furthermore, we have observed in preliminary experiments that bronchoconstriction can be triggered with aerosolized FMLP, in agreement with the postulated role for alveolar cells. The failure of indomethacin to inhibit bronchoconstriction rules out a major causative role of cyclo-oxygenase-dependent metabolites in FMLP-induced bronchoconstriction *in vivo*, and accordingly aspirin's effectiveness must be explained by another mechanism. This cannot involve a salicylic acid-dependent effect, because the latter drug also failed to interfere with the effects of FMLP. On the other hand, aspirin has been found to inhibit lipoxygenases (Siegel *et al.*, 1979; Paajanen *et*

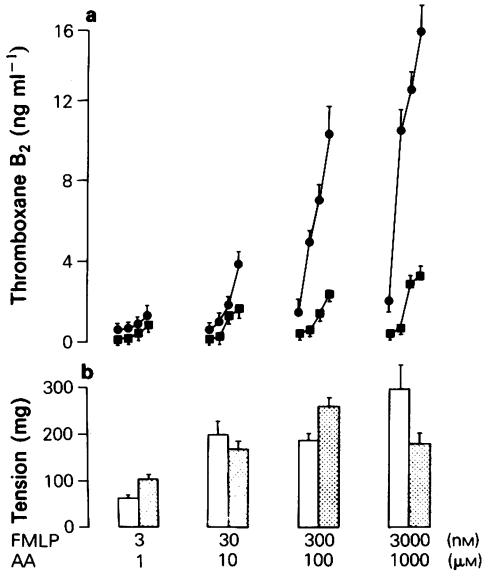

Figure 8 Thromboxane B_2 release (a) and contraction of guinea-pig lung strips (b) induced by arachidonic acid (AA, ● and cross-hatched columns, $n = 4$) and by *N*-formyl methionyl leucyl phenylalanine (FMLP, ■ and open columns, $n = 4$). Vertical lines indicate s.e.mean.

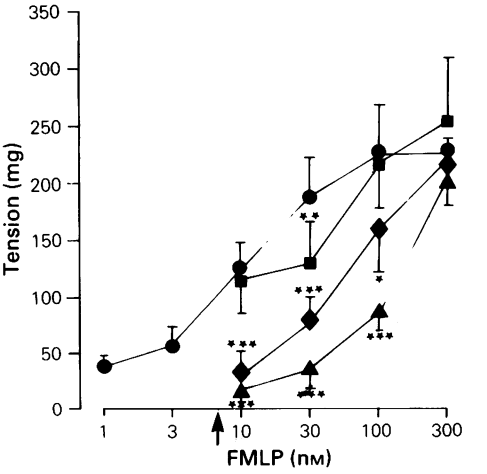

Figure 9 Effect of tert-butyloxy-carbonyl-L-methionyl-L-leucyl-L-phenylalanine (BOC) 3 (■), 10 (◆) and 30 (▲) μM on the contraction of guinea-pig lung strips induced by *N*-formyl methionyl leucyl phenylalanine (FMLP). (●) Indicates the control responses, vertical lines show s.e.mean. BOC was added as indicated by the arrow, $n = 4$. *P < 0.05, **P < 0.01, ***P < 0.001.

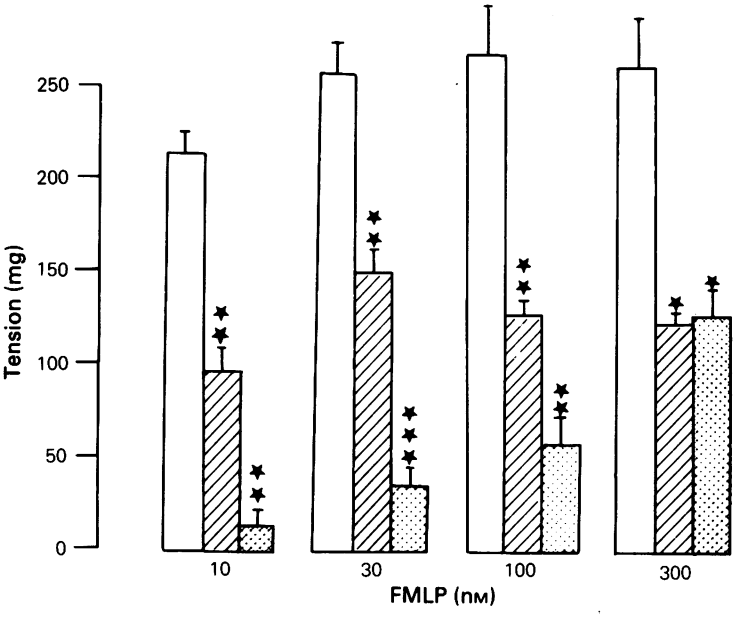

Figure 10 Effect of aspirin (0.1 mM) alone (diagonally-hatched columns, $n = 4$) and aspirin (0.1 mM) plus FPL 55712 (20 mM) (cross-hatched columns, $n = 4$) on the contraction of the guinea-pig lung strips induced by *N*-formyl methionyl leucyl phenylalanine (FMLP). Vertical lines indicate s.e.mean. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Open columns represent control responses to FMLP.

al., 1982; Shulz *et al.*, 1986), and this might explain its activity against FMLP. The effectiveness of the mixed cyclo-oxygenase/lipoxygenase inhibitor compound BW 755C (Anderson *et al.*, 1983) in blocking FMLP-induced bronchoconstriction supports the assumption that lipoxygenase metabolites of arachidonic acid are

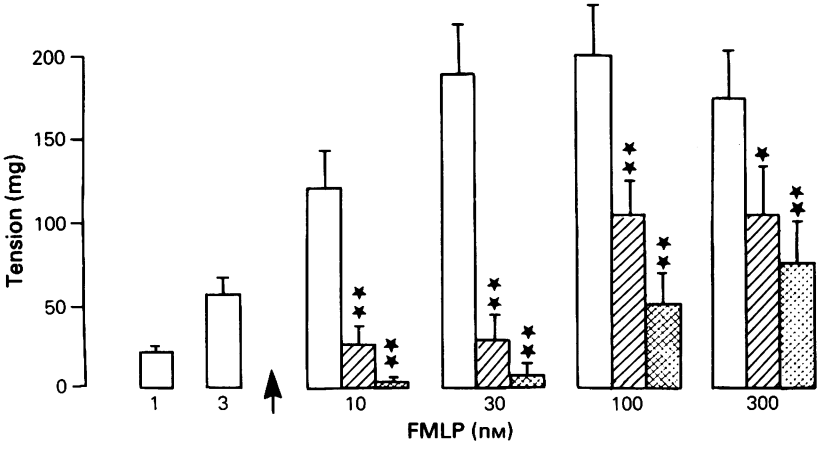

Figure 11 Effect of indomethacin alone (diagonally-hatched columns, 1 μM, $n = 7$) and indomethacin (1 μM) plus FPL 55712 (20μM) (cross-hatched columns, $n = 4$) on the contraction of the guinea-pig lung strips to *N*-formyl methionyl leucyl phenylalanine (FMLP). Vertical lines indicate s.e.mean and the arrow indicates administration of the antagonists. $*P < 0.01$, $**P < 0.001$.

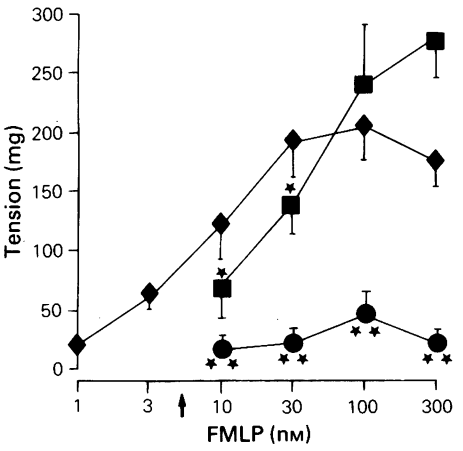

Figure 12 Effect of BW 755C (●, 100 μM, $n = 4$) and FPL 55712 (■, 20 μM, $n = 4$) on the contraction of the guinea-pig lung strips induced by *N*-formyl methionyl leucyl phenylalanine (FMLP). Antagonists were added as indicated by the arrow. Vertical lines indicate s.e.mean. $*P < 0.05$, $**P < 0.001$. (◆) Indicates the control responses.

involved, but the failure of two antagonists to inhibit bronchoconstriction induced by i.v. peptido-leukotrienes (Vargaftig *et al.*, 1981a) argues against their involvement. Furthermore, since BW 755C also inhibited arachidonic acid-induced bronchoconstriction (not shown), which is TXA_2-dependent, its effectiveness cannot be regarded as evidence for the involvement of lipoxygenase.

Because of the complications of the *in vivo* situation, studies were performed on isolated parenchymal lung strips. It is unlikely that PMNL participate in the contractions of these preparations, since the tissues were thoroughly washed to remove contaminant cells. Since tert-butyloxy-carbonyl-L-methionyl-L-leucyl-L-phenylalanine was a competitive antagonist of FMLP *in vitro*, contractions of this tissue are probably triggered by a receptor-dependent event, on an as yet uncharacterized cell component. Contractions due to FMLP were concentration-dependent, and accompanied by the synthesis of TXB_2, which amounted approximatively to 1/6th of that obtained with an equieffective concentration of arachidonate. In both instances, aspirin and indomethacin suppressed the formation of TXB_2 (not shown). The difference between arachidonic acid and FMLP was that the two cyclo-oxygenase inhibitors suppressed TXB_2 formation triggered by both compounds but only totally suppressed the tissue contractions induced by arachidonic acid; a large part of the contractile effect of FMLP persisted. This suggests that cyclo-oxygenase-independent mediators also account for the *in vitro* effects of FMLP. The addition of FPL 55712 (Augstein *et al.*, 1973) increased the aspirin-induced inhibition of the effects of 10, 30 and 100 nM FMLP, but those of 300 nM were not modified (Figure 10). The higher doses of FMLP thus trigger aspirin and FPL 55712-resistant contractions, confirming *in vitro* the *in vivo* observations that the cyclo-oxygenase-independent component is not attributable to peptido-leukotrienes which are antagonized by FPL 55712. Finally, since BW 755C alone inhibited totally the contractile activity of FMLP (Figure 12), it is likely that the aspirin-resistant contraction involves lipoxygenase-dependent mechanisms other than those which are inhibited by FPL 55712 i.e., which are accounted for by the formation of leukotrienes C_4 and D_4.

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