Paf-induced release of spasmogens from guinea-pig lungs

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- 1 The injection of platelet activating factor (Paf; 250 ng), leukotriene B₄ (LTB₄; 50 ng) and leukotriene D₄ (LTD₄; 10 ng) elicited contractions of strips of guinea-pig trachea, bronchus and lung parenchyma.
- 2 When the effluent of perfused guinea-pig lungs was superfused over strips of guinea-pig trachea, bronchus and parenchyma, the intra-arterial injection of Paf (250 ng) caused the release of spasmogen(s) which contracted all three tissues.
- 3 The infusion of indomethacin (10 µg ml⁻¹) into the pulmonary artery and over the assay tissues inhibited the responses of the tissues to the effluent of the lungs stimulated by Paf (250 ng) and LTB₄ (50 ng). However, treating only the assay tissues with indomethacin (10 µg ml⁻¹) did not block the contractile responses to the effluent of the lungs stimulated with LTB₄ or Paf.
- 4 Pretreatment of the lungs with indomethacin (10 µg ml⁻¹) or aspirin (30 µg ml⁻¹) for 30 min, washing them out and suspending them over the assay tissues did not block the release of spasmogens elicited by Paf but appeared to inhibit the release of cyclo-oxygenase products.
- 5 The infusion of two lipoxygenase inhibitors, nordihydroguaiaretic acid (NDGA; 1 µg ml⁻¹) and L-655,240 (1 µg ml⁻¹), into the pulmonary artery completely blocked the release of spasmogen(s) from the perfused lungs.
- 6 The slow reacting substance of anaphylaxis (SRS-A) antagonist, FPL-55712 (10 ng ml⁻¹), did not block the responses of the tissues to the spasmogen(s) released by Paf.
- 7 The infusion of the Paf antagonist BN-52021 (30 µg ml⁻¹) into the pulmonary artery completely abolished the release of spasmogen(s) induced by Paf.
- 8 These data suggest that a lipoxygenase product, possibly LTB₄, could be responsible for the spasmogenic activity released by the lungs following Paf stimulation. Cyclo-oxygenase products released following Paf stimulation appear to result from the initial LTB₄ generation.

Introduction

Experimental evidence suggests that platelet activating factor (Paf) is an important mediator of allergen-induced asthma (Page *et al.*, 1984; Vargaftig & Braquet, 1987). Intravenous injection of Paf induces a strong bronchoconstriction (Vargaftig *et al.*, 1980) which does not seem to be induced by a direct effect of Paf on bronchus (Jancar *et al.*, 1987). We have recently shown that Paf has no effect on preparations of guinea-pig lower bronchus whereas it

induces a contraction of the upper bronchus which is followed by relaxations to subsequent injections of Paf. However, when Paf was injected into isolated perfused lungs, it stimulated the release of substance(s) causing contractions of bronchus, trachea and parenchyma strips (Jancar *et al.*, 1987). Intravenous injection of Paf into guinea-pigs was shown to induce a platelet-dependent bronchoconstriction which is not mediated by cyclo-oxygenase metabolites (Vargaftig *et al.*, 1980; 1982) and is inhibited by several lipoxygenase inhibitors (Bonnet *et al.*, 1983). Aerosol administration of Paf in guinea-pigs was also shown to induce bronchoconstriction that is blocked by aspirin (Maridonneau-Parini *et al.*, 1985). In *in vitro* experiments on guinea-pig lungs

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perfused with cell-free solutions, the injection of Paf induced a bronchoconstriction that was platelet-independent and blocked by cyclo-oxygenase inhibitors. It was also shown that Paf induced a release of thromboxane A_2 (TxA_2) which preceded the increase in airway and pulmonary pressures (Lefort *et al.*, 1984). Thus, the effects of Paf in the lungs appear to depend on the route of administration, which probably reflects the stimulation of different cell types.

The present study represents an attempt to understand better the mechanism of action of Paf in the lung. Guinea-pig isolated lungs were perfused via the pulmonary artery and the effluent directed over strips of guinea-pig trachea, bronchus and parenchyma. After desensitization of the assay tissues to the direct effects of Paf, the lipid mediator was injected into the pulmonary artery circulation and the response of the tissues recorded. To investigate the nature of the spasmogens released, the lungs or the tissues were treated with aspirin and indomethacin, inhibitors of cyclo-oxygenase, nordihydroguaiaretic acid (NDGA), an inhibitor of lipoxygenases, L-655,240, an inhibitor of 5-lipoxygenase and an antagonist of thromboxane receptors, FPL-55712 and BN-52021, antagonists of the peptidoleukotrienes and Paf respectively, and a mixture of antagonists of histamine receptors, cholinceptors and β -adrenoceptors.

Methods

Dunkin Hartley guinea-pigs weighing 300–400 g were killed by cervical dislocation. The thorax was cut open and the trachea, lungs and heart were removed and put in cold Krebs solution. Strips of lung parenchyma ($3 \times 3 \times 30$ mm) were cut along the edges of the lobes according to the technique described earlier (Sirois *et al.*, 1980). The trachea was removed, cut in spirals and divided into three equal segments. Strips of bronchus were prepared as described previously (Sirois *et al.*, 1987b). Briefly, a metal rod was inserted in the trachea and right bronchus. The parenchyma around the main bronchus was dissected and the right and left bronchi were then cut from the trachea. With a smaller metal rod used as support, the bronchi were further dissected free of parenchyma. The tissues were then cut in spirals of approximately 1.5 mm width and 1.5 cm long. Each bronchus was divided into two segments and the upper (external) part was used. It is referred to as GPB-EXT.

The tissues were placed in the organ baths of a cascade superfusion system and perfused with oxygenated (95% O_2 :5% CO_2) Krebs solution (5 ml min^{-1} ; 37°C). The agonists were given as bolus injections (10 – $50 \mu\text{l}$) into the superfusion fluid and

the responses were recorded with Grass FT03C isometric transducers coupled to a Grass polygraph.

The guinea-pig lungs were perfused as described previously (Engineer *et al.*, 1978). In brief, guinea-pigs were killed by cervical dislocation and the lungs were dissected and transferred to a perfusion apparatus where they were perfused with gassed Krebs solution via the pulmonary artery. The lungs were initially perfused at a rate of 10 ml min^{-1} for about 5 min to hasten blood clearance and then at 5 ml min^{-1} . The effluent of the lungs was directed over strips of guinea-pig trachea, bronchus and lung parenchyma.

Drugs used

Histamine hydrochloride, atropine sulphate and indomethacin were purchased from Sigma Chemical Co. (St. Louis, USA); Paf $C_{16}(1\text{-}O\text{-hexadecyl-2-}O\text{-acetyl-sn-glycero-3-phosphorylcholine})$ from Bachem (Switzerland); methysergide bimalate (Sandoz Pharm., Montreal); propranolol hydrochloride (Ayerst Lab., Montreal); phenoxybenzamine hydrochloride (Smith Kline & French, Montreal); diphenhydramine hydrochloride (Parke Davis, Montreal); leukotriene D_4 methyl ester was kindly supplied by Dr S. Rakhit from Biomega (Montreal) and LTD_4 (free acid) by Dr J. Rokach (Merck Frosst Lab., Montreal). LTD_4 methyl ester was hydrolysed with a 5% solution of Na_2CO_3 for 1 h. Paf was dissolved in saline solution containing bovine serum albumin (0.25%), divided into aliquots and kept frozen at -20°C . Before each experiment, it was diluted to an appropriate concentration with Krebs solution and used immediately. Compounds BN-52021 and L-655,240 are 3-*t*-butyl-hexahydro-4,7b,11-trihydroxy-8-methyl-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 and (3-[1-(4-chlorobenzyl)-5-fluoro-3-methyl-indol-2-yl]2,2-dimethylpropanoic acid) respectively (Braquet *et al.* 1987; Hall *et al.* 1987).

Results

Release of spasmogens from guinea-pig isolated lung by leukotrienes B_4 , D_4 and Paf

Guinea-pig isolated lungs were perfused via pulmonary artery and the effluent directed over strips of guinea-pig trachea (GPT), upper external bronchus (GPB-EXT) and lung parenchyma (GPLPS). After a first injection of Paf (250 ng) over the assay tissues to record the initial responses, they were submitted to a continuous infusion of Paf (50 ng ml^{-1}) in order to desensitize them to the direct effect of this agonist. As shown in Figure 1, the first injection of Paf following the initiation of the

Figure 1 Release of spasmogens from perfused guinea-pig lungs stimulated by Paf. Paf (250 ng) was injected as a bolus several times either directly (dir) over strips of guinea-pig trachea (GPT), external bronchus (GPB-EXT) and lung parenchyma (GPLPS) or injected in the pulmonary circulation (i.a.) and the effluent was superfused over the tissues. The initial injection (dir) induced characteristic contractions of the three tissues. The continuous direct infusion of Paf (50 ng ml⁻¹) produced a desensitization of the isolated tissues as indicated by the absence of effect of the second direct injection of Paf. The injection of Paf (250 ng; third panel) into the pulmonary artery (i.a.) stimulated the release of spasmogens and the contraction of the isolated tissues. The fourth and fifth panels show the responses of the tissues to direct injections of leukotriene B₄ (LTB₄, 50 ng) and LTD₄ (10 ng). The last panel shows the effect of an intra-arterial injection of LTB₄ (50 ng). This experiment was repeated at least 3 times.

infusion did not elicit a response on any of the assay tissues. After these procedures, Paf (250 ng) and LTB₄ (50 ng) were injected into the pulmonary circulation (i.a.) and the responses of the assay tissues to the effluent of the stimulated lungs were recorded. Figure 1 shows that injections of Paf and LTB₄ into the lungs induced the release of substance(s) that contracted all three tissues. Direct injections (dir) of LTB₄ (50 ng) and LTD₄ (10 ng) over the assay tissues, made for control purposes, elicited contractions despite the desensitization to Paf.

Characterization of the spasmogenic activity present in the effluent of Paf-treated lungs

In a series of experiments, the assay tissues were continuously treated with a mixture of antagonists containing (μg ml⁻¹): methysergide (0.2), propranolol (3.0), phenoxybenzamine (0.1), atropine (0.1), and diphenhydramine (0.1). The lungs were suspended over the tissues and injected with 250 ng of Paf. These experiments were done to insure that the myotropic activity released from the lungs stimulated

with Paf cannot be ascribed to histamine, 5-hydroxytryptamine, catecholamines or acetylcholine. The assay tissues treated with the mixture of inhibitors contracted to the effluent from Paf-stimulated lungs as the non-treated tissues (data not shown).

Effect of indomethacin and aspirin on Paf-stimulated release of spasmogens

In these experiments, the effect of indomethacin (10 μg ml⁻¹) was studied on the release of spasmogens by the lungs. As shown in Figure 2, the initial injection of Paf (250 ng) directly (dir) over the guinea-pig trachea, bronchus and parenchyma elicited strong contractions. For the remainder of the experiments, the assay tissues and the lungs received a continuous infusion of indomethacin (10 μg ml⁻¹); an infusion of Paf (50 ng ml⁻¹) over the tissues was also carried out to produce a complete desensitization. The subsequent injection of Paf (250 ng) into the lungs did not elicit contractions of assay tissues. The response of the tissues to the effluent from lungs stimulated with LTB₄ was also abolished (Figure 2). It can also be seen in this Figure that the

Figure 2 Release of spasmogens from perfused guinea-pig lungs stimulated by Paf and its inhibition by indomethacin. The injections of Paf were made as in Figure 1. Paf (50 ng ml^{-1}) was infused directly together with an infusion of indomethacin (Indo, i.a., $10 \mu\text{g ml}^{-1}$) to block the release of prostaglandins and thromboxanes and the responses of the tissues to Paf. The injection of Paf (250 ng) in the pulmonary circulation (third panel) had no effect. The direct effect of leukotriene B_4 (LTB_4 , 50 ng) was abolished because the response is mediated by the formation of cyclo-oxygenase products (fourth panel) whereas that to LTD_4 remained (fifth panel). The last panel shows that the intra-arterial injection of LTB_4 (50 ng) did not release prostaglandins and thromboxanes from the lungs. This is a representative experiment that was repeated at least three times.

contractions of the three tissues to the direct injection of LTB_4 were abolished by indomethacin. Contractions of the tissues to LTD_4 were reduced but not blocked by this treatment. It is not possible to conclude from this experiment whether release of mediator(s) from the lungs was inhibited by indomethacin, or whether the response of the tissues to the spasmogen released was blocked by this treatment. In an attempt to answer this question, two sets of experiments were done. First, indomethacin ($10 \mu\text{g ml}^{-1}$) was infused over the assay tissues only (Figure 3). The tissues were also perfused with Paf (50 ng ml^{-1}) to obtain desensitization. An intra-arterial injection of Paf (250 ng) elicited the release of spasmogens which were detected on the assay tissues. The tissues were no longer responsive to LTB_4 , the action of which is mediated by prostaglandins and thromboxanes, but were stimulated by LTD_4 . LTB_4 (50 ng) (i.a.) also stimulated the release of contractile substances from the perfused lungs.

In the other set of experiments, the lungs were infused with either indomethacin ($10 \mu\text{g ml}^{-1}$) or aspirin ($30 \mu\text{g ml}^{-1}$) and the effluent was not directed to the assay tissues. After 30 min, the infusion of

cyclo-oxygenase inhibitors was stopped, the lungs were washed for 10–15 min and were then suspended over the assay tissues. Again Paf was infused over the tissues for the entire experiment to achieve complete desensitization. Following the injection of Paf into the pulmonary circulation, the effluent induced contractions of the assay tissues (Figure 4). Aspirin-like drugs did not block the release of the spasmogenic substance(s) from Paf-stimulated lungs. The tissues contracted to the direct injection of LTB_4 . The intra-arterial injection of LTB_4 elicited contractions of the target tissues which were equivalent to those produced by the direct injection.

Effect of nordihydroguaiaretic acid (NDGA) on Paf-stimulated release of spasmogens

In an experimental design similar to that of the previous series of experiments, the perfused lungs were treated with NDGA, an inhibitor of lipoxygenases ($1 \mu\text{g ml}^{-1}$). As in the above experiments, the assay tissues were desensitized to Paf. Figure 5 shows that the injection of Paf in the pulmonary circulation did not release detectable amounts of spasmogens in the

Figure 3 Release of spasmogens from perfused guinea-pig lungs stimulated by Paf and control effects of indomethacin on the target tissues. The injections of Paf were done as in Figure 1. A continuous direct infusion of Paf (50 ng ml⁻¹) was carried out together with an infusion of indomethacin (10 μg ml⁻¹) directly over assay tissues. The injection of Paf (250 ng) in the pulmonary circulation (third panel) stimulated the release of spasmogens and the contraction of the isolated tissues. The direct effects of leukotriene B₄ (LTB₄, 50 ng; fourth panel) were completely abolished whereas the direct effects of LTD₄ (10 ng; fifth panel) were not. The last panel shows that the intra-arterial injection of LTB₄ (50 ng) released cyclo-oxygenase products from the lungs. This is a representative experiment that was repeated at least 3 times.

Figure 4 Release of spasmogens from perfused guinea-pig lungs and the effect of aspirin. The injections of Paf were made as in Figure 1. Paf (50 ng ml⁻¹) was infused continuously directly over the assay tissues. The lungs were pretreated for 30 min with aspirin (30 μg ml⁻¹) and the effluent was not directed over the assay tissues. The lungs were then washed for 10–15 min and suspended over the assay tissues. The injection of Paf (250 ng; third panel) in the pulmonary artery (i.a.) stimulated the release of spasmogens and the contraction of the isolated tissues. The fourth and fifth panels show the responses of the tissues to a direct (dir) and to an intra-arterial (i.a.) injections of leukotriene B₄ (LTB₄, 10 ng). This experiment was repeated four times.

Figure 5 Effect of nordihydroguaiaretic acid (NDGA) on the release of spasmogens from perfused guinea-pig lungs stimulated by Paf. The injections of Paf were made as in Figure 1. Paf (50 ng ml^{-1}) was infused continuously together with an infusion of NDGA ($1 \mu\text{g ml}^{-1}$) into the pulmonary circulation to block the lipoxygenase. The injection of Paf (250 ng) into the pulmonary artery (i.a.; third panel) did not stimulate the release of spasmogens as shown by the absence of contraction of the target tissues. Direct injections of leukotriene B_4 (LTB_4 , 50 ng) and LTD_4 (10 ng) elicited contractions of all three tissues (fourth and fifth panels respectively). The last panel shows that the intra-arterial injection of LTB_4 (50 ng) released cyclo-oxygenase products from the lungs. This is a representative experiment that was repeated at least 3 times.

presence of this drug. However, the tissues remained totally responsive to leukotrienes B_4 and D_4 and the lungs maintained their ability to release prostaglandins and thromboxanes following LTB_4 stimulation as shown by the marked contractions of the trachea, bronchus and parenchyma (last right hand side tracings).

Effect of compound L-655,240 on Paf-stimulated release of spasmogens

This set of experiments was designed as the above series. The compound L-655,240 a substance described as a thromboxane antagonist (Hall *et al.*, 1987) which also has definite inhibitory properties against lipoxygenase, was given into the pulmonary artery at a concentration of $1 \mu\text{g ml}^{-1}$. As predicted from the results with NDGA, the injection of Paf into the pulmonary circulation did not lead to the release of spasmogens. As shown in Figure 6, injection of LTB_4 induced the release of substances which contracted the trachea, bronchus and parenchyma.

Effect of FPL-55712 on Paf-stimulated release of spasmogens

The next series of experiments was then planned as an initial attempt to characterize the lipoxygenase products released by lungs following Paf treatment. Control injections of Paf induced contractile responses of the guinea-pig trachea, bronchus and parenchyma. However, following the infusion of an antagonist of slow reacting substance of anaphylaxis (SRS-A) and LTD_4 , FPL-55712 (10 ng ml^{-1}), together with Paf in order to prevent the responses of the tissues to Paf, the injection of Paf (as well as that of LTB_4) in the pulmonary artery induced the release of spasmogens (Figure 7).

Effect of compound BN-52021 on Paf-stimulated release of spasmogens

In this last set of experiments, the tissues were desensitized to Paf, and a Paf antagonist, BN-52021, was

Figure 6 Effect of compound L-655,240 on the release of spasmogens from perfused guinea-pig lung stimulated by Paf. The injections of Paf were made as in Figure 1. Paf (50 ng ml^{-1}) was infused continuously together with an infusion of compound L-655,240 ($1 \mu\text{g mg}^{-1}$) in the pulmonary circulation to block the effects of thromboxane A_2 and the lipoxygenases. The injection of Paf (250 ng) into the pulmonary artery (i.a.; third panel) did not stimulate the release of spasmogens as shown by the absence of contraction of the target tissues. Direct injections of leukotriene B_4 (LTB_4 , 50 ng) and LTD_4 (10 ng) elicited contractions of all three tissues (fourth and fifth panels respectively). The last panel shows that the intra-arterial injection of LTB_4 (50 ng) released spasmogens with reduced activity on the target tissues. This is a representative experiment that was repeated at least 3 times.

infused in the lung to block the receptors to Paf and study the specificity of the lung responses. As shown in Figure 8, the injection of Paf into the lungs did not generate the release of spasmogenic mediators. However, the lungs remained fully responsive to leukotrienes and released substances that contracted all three tissues.

Discussion

These results show that injection of Paf into the pulmonary circulation induces the release of substance(s) that contract strips of trachea, bronchus and parenchyma. These contractions cannot be attributed to Paf itself since the tissues were shown to be desensitized to the direct effects of Paf by its continuous infusion over the tissues. The contractions are not mediated by 5-hydroxytryptamine, catecholamines, acetylcholine or histamine since they were not affected by a mixture of antagonists to these substances. Pretreatment of the lungs with indomethacin or aspirin did not block the generation of the contractile substance(s), which indicates that

the spasmogen is not a cyclo-oxygenase metabolite. The compound L-655,240 which is a thromboxane antagonist and a 5-lipoxygenase inhibitor (Hall *et al.*, 1987; Borgeat, personal communication) and NDGA, a lipoxygenase inhibitor blocked the release of the contractile substance(s). The peptido-leukotriene antagonist, FPL-55712, did not block the contractile response of the tissues to the spasmogen released. Thus a 5-lipoxygenase product, possibly LTB_4 could be the main spasmogen generated in the lungs following stimulation with Paf. This assumption is corroborated by the experiments where the lungs as well as the assay tissues were perfused with indomethacin. In this case, the contractions of the assay tissues were completely abolished. It is known that the contraction induced by LTB_4 in pulmonary tissues is mediated by cyclo-oxygenase products (Sirois *et al.*, 1982). Although the generation of LTB_4 in the lungs is not affected by indomethacin treatment, the effect of LTB_4 on the assay tissues should be blocked by indomethacin. An apparent contradiction appears when analysing the results obtained when the tissues but not the lungs were treated with indomethacin. This treatment did not affect the contractions of the assay tissues to the effluent from Paf-

Figure 7 Effect of compound FPL-55712 on the release of spasmogens from perfused guinea-pig lungs stimulated by Paf. The injections of Paf were made as in Figure 1. Paf (50 ng ml^{-1}) was infused continuously together with compound FPL-55712 ($10 \mu\text{g ml}^{-1}$) directly over the assay tissues. The injection of Paf (250 ng) in the pulmonary artery (third panel) stimulated the release of spasmogens and the contraction of the isolated tissues. The direct injection of leukotriene B_4 (LTB_4 , 50 ng; fourth panel) elicited contractions of the tissue whereas that of LTD_4 (10 ng; fifth panel) was completely abolished by the selective leukotriene antagonist. The last panel shows that the intra-arterial injection of LTB_4 (50 ng) released cyclo-oxygenase products from the lungs. This is a representative experiment that was repeated at least 3 times.

Figure 8 Effect of compound BN-52021 on the release of spasmogens from perfused guinea-pig lung stimulated by Paf. The injections of Paf were made as in Figure 1. Paf (50 ng ml^{-1}) was infused continuously together with an infusion of compound BN52021 ($30 \mu\text{g ml}^{-1}$) directly over the assay tissues. The third panel shows that the assay tissues did not react to the injection of Paf (250 ng) in the pulmonary artery. Direct injections of leukotriene B_4 (LTB_4 , 50 ng) and LTD_4 (10 ng) elicited contractions of all three tissues (fourth and fifth panels respectively). The last panel shows that the intra-arterial injection of LTB_4 (50 ng) released cyclo-oxygenase products from the lungs. This is a representative experiment that was repeated at least 3 times.

stimulated lungs. In this case the contraction of the assay tissues cannot be attributed to LTB_4 since indomethacin abolished the response of the tissues to the direct injections of LTB_4 . One possible explanation for these results is that Paf induces release of LTB_4 which in turn produces thromboxane, both steps occurring in the lungs. The thromboxane released in the lung effluent then induced a contraction of the assay tissues regardless of the presence of indomethacin.

Further evidence in support of this hypothesis is provided by the similar effects of LTB_4 and Paf injection into the pulmonary circulation and by the series of experiments showing that pretreatment of the lungs (not the tissues) with cyclo-oxygenase inhibitors aspirin (Figure 4) and indomethacin did not abolish the release of spasmogens. In accordance with this assumption are the data obtained by Lefort *et al.* (1984) who found that the bronchoconstriction induced by Paf injection into isolated perfused guinea-pig lungs was inhibited by aspirin. Furthermore, they have found that the bronchoconstriction was inhibited by NDGA and mepacrine but not by FPL-55712. These results support the hypothesis that generation of LTB_4 is a prerequisite for the effects of Paf in the lung. Indeed, Paf induces the release of considerable amounts of LTB_4 (Sirois *et al.*, 1987a) as well as thromboxane A_2 (Lefort *et al.*, 1984; Hamasaki *et al.*, 1984; Sirois *et al.*, 1987a) from isolated guinea-pig lungs and polymorphonuclear leukocytes (Chilton *et al.*, 1982; Lin *et al.*, 1982). Our results are also in agreement with those of Hamasaki *et al.* (1984) which showed that the increase of airway pressure in isolated perfused lungs of guinea-pig stimulated with Paf is inhibited by indomethacin.

The situation *in vivo* seems to be different. In this case, the bronchoconstriction induced by intravenous injection of Paf is not affected by cyclo-

oxygenase inhibitors (Vargaftig *et al.*, 1980; 1982), although the bronchoconstriction induced by aerosol administration of Paf is inhibited by aspirin (Maridonneau-Parini, 1985). Since there are some discrepancies between the direct effects of Paf on guinea-pig bronchus and the bronchoconstriction induced by Paf *in vivo* (Jancar *et al.*, 1987), we believe that the *in vivo* action of Paf may represent an indirect effect via activation of platelets, neutrophils, vascular endothelial cells or other cell types. What cell type is the target for Paf-induced release of spasmogens in the lung is yet to be determined.

The different results obtained with various routes of administration of Paf can be explained by assuming that different spasmogens are released depending on the cell type that is first stimulated by Paf. Alveolar macrophages may be the first targets when Paf is administered in aerosol, whereas platelets and PMN leukocytes might be stimulated first when Paf is injected intravenously. In the isolated perfused lung model, none of these cells is likely to be involved although macrophages could be reached by diffusion.

It is also interesting to speculate about the biological significance of LTB_4 release in the lung. Morphological studies have shown a marked PMN leukocyte infiltration in the lungs following intravenous injection of Paf (Lellouch-Tubiana, 1985). Accumulation of these cells in the lung parenchyma and alveoli may be due to the secondary formation and to the effects of LTB_4 and may contribute to the tissue lesions in allergic respiratory diseases.

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