Role of eicosanoids in PAF-induced increases of the vascular permeability in rat airways

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1 Platelet activating factor (PAF; 1.0 and 5.0 $\mu\text{g kg}^{-1}$) injected in the tail vein of unanaesthetized rats dose-dependently increased the vascular permeability of the trachea, upper and lower bronchi (up to 400%) as measured by the extravasation of Evans blue dye. The permeability of the parenchyma was not affected by PAF treatment.

2 Pretreatment of the animals with an intravenous injection of the PAF antagonist BN-52021 (10 mg kg^{-1}) abolished almost totally the vascular permeability changes elicited by PAF injection (5.0 $\mu\text{g kg}^{-1}$).

3 Pretreatment of the animals with intravenous injections of inhibitors of thromboxane formation, indomethacin (10 mg kg^{-1}) and compound OKY-046 (10 mg kg^{-1}), and thromboxane antagonist, compound L-655,240 (5 mg kg^{-1}), partially reduced PAF effects in the airways (from 28 to 69%). The thromboxane mimic U-44069 (5.0 $\mu\text{g kg}^{-1}$) did not modify the vascular permeability of rat airways. The effect of a low dose of PAF (0.1 $\mu\text{g kg}^{-1}$) on the vascular permeability of the trachea and bronchi (but not of the parenchyma) was potentiated by compound U-44069 (5.0 $\mu\text{g kg}^{-1}$) or noradrenaline (400 ng kg^{-1}) whereas the effect of a high dose of PAF (5.0 $\mu\text{g kg}^{-1}$) was not affected.

4 Neither the peptidoleukotriene antagonist MK-571 (10 mg kg^{-1}) nor the 5-lipoxygenase inhibitor, L-663,536 (10 mg kg^{-1}) given before the injection of PAF (5.0 $\mu\text{g kg}^{-1}$) affected the protein extravasation in rat lung tissues.

5 These data suggest that the effect of PAF on rat vascular permeability is partly modulated by thromboxane formation although thromboxanes have no direct effect on the permeability. Thromboxane may act via a vasoconstriction that increases hydrostatic pressure and potentiates the extravasation elicited by PAF effect on endothelial cells.

6 Leukotrienes do not appear to be involved in the changes of rat airway permeability induced by PAF.

Introduction

Platelet activating factor (PAF), a potent inflammatory mediator, induces airway microvascular leakage by an uncharacterized mechanism. In many species, the effect of PAF seems to be dependent on the activation of platelets and polymorphonuclear leukocytes and is most probably mediated by the release of secondary messengers (for a review, see Braquet *et al.*, 1987). Inarrea *et al.* (1984), demonstrated that rat platelets have no specific binding sites for PAF-acether. This finding correlated well with the reports which showed that the effect of PAF in rats is independent of the presence of platelets (Wedmore & Williams, 1981; Pirotzky *et al.*, 1984) and neutrophils (Pirotzky *et al.*, 1984). Dewar *et al.* (1983) and Bjork & Smedegard (1983) confirmed by microscopy that PAF acts on the vascular endothelium, produces disjunction of endothelial cells of post-capillary venules and increases vascular permeability. Bolin *et al.* (1987), showed that fluid movement across the endothelial membrane into the interstitial space is dependent on hydrostatic and osmotic pressure gradients as well as endothelial permeability. However, the studies quoted above did not distinguish whether the extravasation elicited by PAF was secondary to increased hydrostatic forces or leakage resulting from increased permeability.

The aims of the following experiments were: (1) to study the effects of PAF on the vascular permeability of rat airways; (2) to determine the mediatory role of eicosanoids in PAF effects and, (3) to characterize the mechanisms responsible for PAF activity.

Methods

Unanaesthetized male Wistar rats (225–275 g) were used in these experiments. Protein extravasation, a marker of vascular

permeability was evaluated by measuring the extravasation of Evans blue dye (EB). In brief, EB (25 mg ml^{-1} in 0.9% NaCl; 20 mg kg^{-1}) was injected in the caudal vein with increasing doses of PAF. The animals were decapitated and exsanguinated at different times (5, 10, 30 and 60 min). In some experiments, the animals received pretreatment with an antagonist or an inhibitor 5 and 60 min respectively before the PAF injections and were killed 5 min later. In one group, the thromboxane mimic (U-44069) was injected simultaneously with EB and in another group, the animals were given an injection of PAF together with either U-44069 or noradrenaline, and EB; the animals were killed 5 min later. The thorax was cut open and the lungs were perfused with 15 ml of Krebs solution (10 ml min^{-1}) via a cannula inserted in the pulmonary artery to remove the excess of intravascular pulmonary dye. The trachea, upper and lower bronchi and the pulmonary parenchyma were dissected, weighed and a portion of each organ was put in formamide (4 ml g^{-1} wet weight tissue at 20°C for 24 h) while another part was dried at 60°C for 24 h. The upper bronchi corresponded to the parts of the airways that begin at the trachea up to its entry in the lung parenchyma. The lower airways corresponded to major airways surrounded by lung parenchyma which can be easily dissected without magnification.

Evans blue assay

The concentration of EB extracted in formamide was determined by spectrophotometry at 620 nm wavelength using a Titertek Multiskan (Flow Lab.) and 96 well plates. The results were plotted on a standard curve of EB (0.5–25 $\mu\text{g ml}^{-1}$). Evans blue content of each sample was expressed as EB $\mu\text{g g}^{-1}$ dry weight of tissue. The expression of our results as a function of dry weight of tissue avoided underevaluation of changes due to oedema formation.

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Drugs used

The following drugs were used: Evans blue and noradrenaline hydrochloride (Sigma Chem., St Louis, U.S.A.), PAF(1-O-hexadecyl - 2 - O - acetyl - sn - glycerol - 3 - phosphorycholine) (Bachem, Switzerland), U-44069 15(S)-hydroxy-9 α ,11 α -(epoxymethano)-prosta-5-cis,13-trans-dienoic acid (gift from Dr J. Pike, Upjohn, Kalamazoo, U.S.A.), BN-52021 (3-*t*-butyl-hexahydro - 4,7b,11 - trihydroxy - 8 - methyl - 9H - 1,7a - epoxy - methano - 1H,6aH - cyclopenta[c]furo[2,3 - b]furo- [3',2':3,4] cyclopenta[1,2-d] furan-5,9,12(4H) trione; L-655,240 (3-[1-(4-chlorobenzyl)-5-fluoro-3-methyl-indol-2-yl]2,2-dimethyl propanoic acid), MK-571 (3-(3-(2-(7-chloro-2-quinolinyl)ethenyl) phenyl ((3-dimethyl amino-3-oxo propyl) thio) methyl thio) propanoic acid), indomethacin, L-663,536 3-[1-(4-chlorobenzyl)-3-*t*-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid (gifts from Merck Frosst Lab., Montreal, Canada), OKY-046 ((E)-3-[4-(1-imidazolylmethyl) phenyl]-2-propenoic acid hydrochloride monohydrate; gift from ONO Pharmaceuticals, Japan).

Statistical analysis

Results are expressed as means $\pm$ s.e.mean and significance has been determined by analysis of variance. *P* values < 0.05 were considered as significant.

Results

Effects of PAF on protein extravasation in rat airways

In the first series of experiments, EB dye was injected with increasing doses of PAF (1.0 and 5.0 $\mu\text{g kg}^{-1}$) in the caudal vein of rats that have been killed 5, 10, 30 and 60 min after the injection. A group of rats received EB only and were used as controls. The following organs were collected: trachea, upper and lower bronchi and the pulmonary parenchyma. As shown in Figure 1, basal levels of EB (control) were fairly stable as a function of time and ranged from 50 to 140 $\mu\text{g g}^{-1}$ of dry weight in all tissues. A slight increase as a function of time was noted in the pulmonary parenchyma. The injection of PAF (1.0 $\mu\text{g kg}^{-1}$) increased the content of EB in the trachea, upper and lower bronchi by 99%, 170% and 156% respectively, and the maximal effect was noted at 60 min. The highest concentration of PAF (5.0 $\mu\text{g kg}^{-1}$) increased the EB extravasation by 204%, 755% and 397% in the same three tissues. The maximal effect was reached at 5 min (Figure 1). PAF injections up to 5.0 $\mu\text{g kg}^{-1}$ did not affect the permeability of lung parenchyma (Figure 1b).

Effects of the PAF antagonist BN-52021 on PAF-induced vascular permeability

In the second series of experiments, the effects of the PAF antagonist BN-52021 (10 mg kg^{-1}) were studied on the vascular permeability changes elicited by PAF injection to conscious rats. The antagonist was injected in the tail vein 5 min before the injection of PAF (5.0 $\mu\text{g kg}^{-1}$) and EB. The animals were decapitated 5 min later. As shown in Figure 2, compound BN-52021 reduced by 80, 92 and 100% the protein extravasation in the trachea, upper and lower bronchi, respectively. Compound BN-52021 did not affect the level of permeability of the parenchyma. Given in the absence of PAF, this antagonist did not modify either the basal protein extravasation of the trachea, upper and lower bronchi.

Effects of selected antagonists and inhibitors of thromboxane formation

The present series of experiments was undertaken to evaluate the role of thromboxane A_2 in the lung response to PAF and to determine the role of constriction in the increase of vascular

Figure 1 Evans blue dye contents ($\mu\text{g g}^{-1}$ dry tissue) as a function of time after the intravenous injection of increasing concentrations of PAF. (□) Control, (▨) 1.0 $\mu\text{g kg}^{-1}$, (■) 5.0 $\mu\text{g kg}^{-1}$ together with Evans blue dye (20 mg kg^{-1}). * *P* < 0.05 and ** *P* < 0.01 as compared with controls in the absence of PAF. Columns are means of 4–6 experiments; vertical lines show s.e.mean.

permeability. As shown in Figure 3, the treatment of rats with indomethacin (10 mg kg^{-1}), an inhibitor of prostaglandin and thromboxane formation, injected 60 min before PAF (5.0 $\mu\text{g kg}^{-1}$), reduced by 49, 28 and 46% protein extravasation in the trachea, upper and lower bronchi respectively. No significant effect was noted in the parenchyma.

The compound OKY-046 (10 mg kg^{-1}), a thromboxane synthetase inhibitor injected 60 min before the PAF injection (5.0 $\mu\text{g kg}^{-1}$), reduced by 69 and 36% the protein extravasation of the trachea and upper bronchus respectively but did not affect significantly PAF-induced vascular permeability of the lower bronchus and parenchyma. Similarly, compound L-655,240 (5 mg kg^{-1}), a novel thromboxane antagonist, injected 5 min before the PAF injection (5.0 $\mu\text{g kg}^{-1}$), reduced

Figure 2 Effects of pretreating the rats with compound BN-52021 (10 mg kg^{-1}) 5 min before a PAF injection (5.0 $\mu\text{g kg}^{-1}$) together with Evans blue dye (20 mg kg^{-1}). The rats were killed 5 min after the PAF treatment. The contents of Evans blue dye ($\mu\text{g g}^{-1}$ dry tissue) are means of 4–6 experiments; vertical lines show s.e.mean. * *P* < 0.05 and ** *P* < 0.01 respectively as compared with PAF.

Figure 3 Effects of pretreating the rats with indomethacin (Indo; 10 mg kg^{-1}), compound OKY-046 (OKY; 10 mg kg^{-1}) and compound L-655,240 (L-240; 5 mg kg^{-1}) on the effect of PAF ($5.0 \mu\text{g kg}^{-1}$). The animals were pretreated with the inhibitors indomethacin and OKY-046 60 min before the injection of PAF and Evans blue dye (20 mg kg^{-1}) whereas the antagonist of thromboxane, compound L-655,240 was given 5 min before the injection of PAF. The rats were killed 5 min after the PAF treatment. The thromboxane mimic (U-44069; $5.0 \mu\text{g kg}^{-1}$) was injected together with Evans blue dye (20 mg kg^{-1}) and the rats were killed 5 min later. Values are means of 4–6 experiments; vertical lines show s.e.mean. * and ** as in Figure 2.

protein extravasation in the trachea and upper bronchi by 33 and 32%, respectively, whereas it did not interfere with the permeability of the lower bronchus and the parenchyma.

Based on these results that suggested a role for thromboxane in PAF-induced protein extravasation, compound U-44069 ($5.0 \mu\text{g kg}^{-1}$), a thromboxane mimic, was used. As shown on the right hand side of each panel of Figure 3, a large dose of this compound ($5 \mu\text{g kg}^{-1}$) did not induce protein extravasation from the selected lung tissues 5 min after its injection.

Figure 4 Effects of compound U-44069 ($5.0 \mu\text{g kg}^{-1}$) or noradrenaline (NA, 400 ng kg^{-1}) combined with a low dose of PAF ($0.1 \mu\text{g kg}^{-1}$) on protein extravasation by rat airways. Evans blue dye (20 mg kg^{-1}) was injected alone as a control. The rats were killed 5 min after the treatment. Values are means of 4–6 experiments; vertical lines show s.e.mean. * and ** as in Figure 2.

Figure 5 Effects of compound U-44069 ($5.0 \mu\text{g kg}^{-1}$) or noradrenaline (NA, 400 ng kg^{-1}) combined with a high dose of PAF ($5.0 \mu\text{g kg}^{-1}$) on protein extravasation by rat airways. Evans blue dye (20 mg kg^{-1}) was injected alone as a control. The rats were killed 5 min after the treatment. Values are means of 4–6 experiments; s.e.mean shown by vertical lines.

Effect of noradrenaline and compound U-44069 on PAF-induced microvascular leakage

In order to understand better the basic mechanisms underlying microvascular leakage and especially the role of vasoconstriction in this process, the effects of noradrenaline and U-44069 on PAF-induced increases in EB extravasation were studied. PAF was used at doses of $0.1 \mu\text{g kg}^{-1}$ and $5 \mu\text{g kg}^{-1}$. As shown in Figure 4, the low dose of PAF produced only slight changes on EB extravasation in airway and lung tissues.

Figure 6 Effects of pretreating rats with compounds L-663,536 (L-536; 10 mg kg^{-1}) and MK-571 (10 mg kg^{-1}) 60 and 5 min respectively before the PAF injection ($5.0 \mu\text{g kg}^{-1}$) together with Evans blue dye (20 mg kg^{-1}). The rats were killed 5 min after the PAF treatment. The content of Evans blue dye ($\mu\text{g g}^{-1}$ dry tissue) are means of 4–6 experiments; s.e.mean shown by vertical lines.

The simultaneous injection of PAF ($0.1 \mu\text{g kg}^{-1}$) and U-44069 ($5 \mu\text{g kg}^{-1}$) increased by 67 and 65% the protein extravasation in the trachea and upper bronchi respectively, as compared with the effect of PAF alone, but did not affect significantly the extravasation in the lower bronchi and parenchyma. Similarly, the combined injection of the low dose of PAF ($0.1 \mu\text{g kg}^{-1}$) and noradrenaline (400 ng kg^{-1}) increased by 57 and 74% the extravasation in the trachea and lower bronchi and did not affect significantly the permeability of the lower bronchi and parenchyma.

Figure 5 shows the effects of noradrenaline and compound U-44069 on the effects of a higher dose of PAF ($5.0 \mu\text{g kg}^{-1}$) on vascular permeability. The results on the effect of PAF are identical to those presented in Figure 1. They show that PAF strongly increased the protein extravasation in the trachea, upper and lower bronchi but not in the parenchyma. Compound U-44069 ($5.0 \mu\text{g kg}^{-1}$) as well as noradrenaline (400 ng kg^{-1}) given in combination with PAF ($5.0 \mu\text{g kg}^{-1}$) did not act in synergism and the total EB extravasation in all 4 tissues was similar to the extravasation produced by the injection of PAF alone.

Effects of an antagonist and an inhibitor of leukotriene formation

In the last series of experiments, the role of leukotrienes as possible modulators of PAF activities was evaluated with the use of two novel molecules: compound L-663,536, a lipoxygenase inhibitor, and compound MK-571, a peptido-leukotriene antagonist. Given at a dose of 10 mg kg^{-1} , 60 and 5 min, respectively, before the injection of PAF, compound L-663,536 and compound MK-571 did not block the increases of protein extravasation in lung tissues (Figure 6).

Discussion

These results show that intravenous injections of PAF dose-dependently increased protein extravasation in rat trachea, upper and lower bronchi, but did not modify that of pulmonary parenchyma. The effect of PAF on protein extravasation was almost totally inhibited with compound BN-52021 pretreatment. These results are in agreement with those of Wedmore & Williams (1981) and Page *et al.* (1983) who demonstrated that PAF increases vascular permeability in rabbit and guinea-pig skin and paw. Evans *et al.* (1986; 1987) and O'Donnell & Barnett (1987) also described PAF-mediated protein extravasation in guinea-pig airways at a dose 1000 times less than in rats. Bjork & Smedegard (1983) suggested that the difference in PAF doses needed to induce an effect in various species is related to platelet receptor sensitivity to this mediator. The absence of effect of PAF noted in the lung parenchyma as compared to the trachea and bronchi could be related to the microcirculation under study. Lung parenchyma is perfused by the pulmonary circulation whereas the tracheal and bronchial tissues are perfused by the systemic circulation.

Our results also show that the thromboxane/prostaglandin endoperoxide antagonist L-655,240, as well as the inhibitors indomethacin and compound OKY-046, decreased the protein extravasation induced by PAF injection. The partial inhibition of the protein extravasation produced either by a pretreatment with a thromboxane antagonist or by inhibitors of thromboxane formation, suggests that intravenous PAF

treatment stimulates thromboxane secretion which potentiates or modulates protein extravasation. However, Bjork & Smedegard (1983) and Evans *et al.* (1987) did not reach the same conclusions since indomethacin did not modify PAF-induced increases in vascular permeability in the hamster cheek pouch and in guinea-pig airways.

The thromboxane mimic, U-44069, was not shown to affect vascular permeability by itself but it potentiated the effect of low dose of PAF. Similarly, noradrenaline potentiated the effects of a low dose of PAF on EB extravasation. These results appear paradoxical. However, vascular permeability is controlled by complex mechanisms which include the opening of permeability channels by disjunction of endothelial cells and increase of capillary hydrostatic pressure. A number of studies have shown that the effects of PAF on vascular permeability are secondary to disjunction of endothelial cells (Dewar *et al.*, 1983; Bjork & Smedegard, 1983; Handley *et al.*, 1984). Heffner *et al.* (1983) showed that PAF induces the release of thromboxanes which raises microvascular pressure. The increment in microvascular pressure does not itself cause changes in vascular permeability since neither compound U-44069 nor noradrenaline, two potent vasoconstrictors, increased vascular permeability in our study. However, it is possible that in the presence of PAF-induced cell disjunction, vasoconstrictors may raise hydrostatic pressure by post-capillary vasoconstriction and consequently, potentiate plasma leakage. Our data, as well as those presented by Heffner *et al.* (1989), strongly support this hypothesis. It appears that vascular permeability is only modulated by changes in blood pressure. For instance, peptido-leukotrienes are vasoconstrictors and increase vascular permeability (Dahlen *et al.*, 1981) while histamine is a potent dilator of the microcirculation but also increases vascular permeability (Majno & Palade, 1961). Drugs acting on cholinergic and adrenoceptors have potent effects on blood pressure but do not affect plasma leakage (reviewed by Grega & Adamski, 1988). Vasoconstrictors such as thromboxane A_2 could potentiate the plasma leakage by an increase of hydrostatic pressure only in the presence of compounds that open the permeability channels.

Neither compound MK-571, a new specific leukotriene D_4 (LTD_4) antagonist (Jones *et al.*, 1989) nor compound L-663,536, a new 5-lipoxygenase inhibitor (Gillard *et al.*, 1989) reduce PAF-induced protein extravasation. These results strongly suggested that the effects of PAF are independent of leukotriene synthesis in rat airways. This is in agreement with the results of Handley *et al.* (1986), who demonstrated that FPL-55712, an antagonist of LTC_4 and LTD_4 , was ineffective in reducing the haemoconcentration mediated by a PAF injection.

In conclusion, intravenous injection of PAF causes massive protein extravasation which is partly mediated by thromboxane secretion. Exogenous thromboxane may potentiate the effects of PAF by producing a vasoconstriction at the post-capillary sites which increases the hydrostatic pressure and potentiates the extravasation. PAF effects seem to be independent of leukotriene generation. The reason for the absence of effect of PAF on the pulmonary parenchyma is under investigation.

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References

- BJORK, J. & SMEDEGARD, G. (1983). Acute microvascular effects of PAF-acether, as studied by intravital microscopy. *Eur. J. Pharmacol.*, **96**, 87–94.
- BOLIN, R.W., MARTIN, T.R. & ALBERT, R.K. (1987). Lung endothelial and epithelial permeability after platelet-activating factor. *J. Appl. Physiol.*, **63**, 1770–1775.
- BRAQUET, P., TOUQUI, L., SHEN, T.Y. & VARGAFTIG, B.B. (1987). Perspectives in platelet-activating factor research. *Pharmacol. Rev.*, **39**, 97–145.
- DAHLEN, S.E., BJORK, J., HEDQVIST, P., ARFORS, K.E., HAMMARSTROM, S., LINDGREN, J.A. & SAMUELSSON, B. (1981). Leukotrienes promote plasma leakage and leukocyte adhesion in

- postcapillary venules: *in vivo* effects with relevance to the acute inflammatory response. *Proc. Natl. Acad. Sci., U.S.A.*, **78**, 3887–3891.
- DEWAR, A., MORLEY, J., PAGE, C.P. & PAUL, W. (1983). Permeability characteristics of platelet activating factor in guinea pig skin. *Br. J. Pharmacol.*, **78**, 180P.
- EVANS, T.W., CHUNG, K.F. & BARNES, P.J. (1986). Platelet-activating factor increases vascular permeability in guinea pig airways. *Clin. Sci.*, **71**, 1P.
- EVANS, T.W., CHUNG, K.F., ROGERS, D.F. & BARNES, P.J. (1987). Effect of platelet-activating factor on airway vascular permeability: possible mechanism. *J. Appl. Physiol.*, **63**, 479–484.
- GILLIARD, J., FORD-HUTCHINGS, A.W., CHAN, C., CHARLESON, S., DENIS, D., FOSTER, A., FORTIN, R., LÉGER, S., MCFARLANE, C.S., MORTON, H., PIECHUTA, H., RIENDEAU, D., ROUZER, C.A., ROKACH, J., YOUNG, R., MACINTYRE, D.E., PETERSON, L., BACH, T., EIERMANN, G., HOPPLE, S., HUMES, J., HUGE, L., LUELL, S., METZGER, J., MEURER, R., MILLER, D.K., OPAS, E. & PACHOLK, S. (1989). L-663,536 (MK-886)(3-[1-(4-chlorobenzyl)-3-t-butylthio-5-isopropylindol-2-yl]-2,2-dimethyl-propanoic acid), a novel, orally active leukotriene biosynthesis inhibitor. *Can. J. Physiol. Pharmacol.*, **67**, 456–464.
- GREGA, G.J. & ADAMSKI, S.W. (1988). The role of venular endothelial cells in the regulation of macromolecular permeability. *Microcirc. Endoth. Lymphatics*, **4**, 143–167.
- HANDLEY, D.A., ARBEENY, C.M., LEE, M.L., VAN VALEN, R.G. & SAUNDERS, R.N. (1984). Effect of platelet-activating factor on endothelial permeability to plasma macromolecules. *Immunopharmacol.*, **8**, 137–142.
- HANDLEY, D.A., FARLEY, C., DEACON, R.W. & SAUNDERS, R.N. (1986). Evidence for distinct systemic extravasation effects of platelet activating factor, leukotrienes B₄, C₄, and D₄ and histamine in the guinea pig. *Prostaglandins, Leukotrienes Med.*, **21**, 269–277.
- HEFFNER, J.E., COOK, J.A. & HALUSKA, P.V. (1989). Human platelets modulate edema formation in isolated rabbit lungs. *J. Clin. Invest.*, **84**, 757–764.
- HEFFNER, J.E., SHOEMAKER, S.A., CANHAM, E.M., PATEL, M., McMURTRY, I.F., MORRIS, H.G. & REPINE, J.E. (1983). Acetyl glyceryl ether phosphorylcholine-stimulated human platelets cause pulmonary hypertension and edema in isolated rabbit lungs. *J. Clin. Invest.*, **71**, 351–357.
- INARREA, P., GOMEZ-CAMBRONERO, J., NIETO, M. & SANCHEZ-CRESPO, M. (1984). Characteristics of the binding of platelet-activating factor to platelets of different animal species. *Eur. J. Pharmacol.*, **105**, 309–315.
- JONES, T.R., ZAMBONI, R., BELLEY, M., CHAMPION, E., CHARETTE, L., HUTCHINSON, A.W., FRENETTE, R., GAUTHIER, J.Y., LEGER, S., MASSON, P., MCFARLANE, C.S., PIECHUTA, H., ROKACH, J., WILLIAMS, H., YOUNG, R.N., DEHAVEN, R.N. & PONG, S.S. (1989). Pharmacology of L-660,711 (MK-571): a novel potent and selective leukotriene D₄ receptor antagonist. *Can. J. Physiol. Pharmacol.*, **67**, 17–28.
- MAJNO, G. & PALADE, G. (1961). Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability: an electron microscopic study. *J. Biophys. Biochem. Cytol.*, **11**, 571–606.
- O'DONNELL, S.R. & BARNETT, C.J.K. (1987). Microvascular leakage to platelet activating factor in guinea pig trachea and bronchi. *Eur. J. Pharmacol.*, **138**, 385–396.
- PAGE, C.P., PAUL, W. & MORLEY, J. (1983). Continuous monitoring of plasma protein extravasation and cell accumulation *in vivo*. *Br. J. Derm.*, **109**, suppl. 25, 103–105.
- PIROTZKY, E., PAGE, C.P., ROUBIN, R., PFISTER, A., PAUL, W., BONNET, J. & BENVENISTE, J. (1984). PAF-acether induced plasma exudation is independent of platelets and neutrophils in the rat skin. *Microcirculation, Endothelium Lymphatics*, **1**, 107–122.
- WEDMORE, C.V. & WILLIAMS, T.J. (1981). Platelet-activating factor (PAF), a secretory product of polymorphonuclear leucocytes, increases vascular permeability in rabbit skin. *Br. J. Pharmacol.*, **74**, 916–917P.

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