The effect of platelet-activating factor on the responsiveness of the human nasal airway

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- 1 The effects of inhaled platelet-activating factor (PAF) on responsiveness of the human nasal airway were examined in normal subjects by measuring nasal airway resistance in response to histamine and bradykinin at 2, 6, 24, 48 h and 7 d after PAF administration. Eosinophil cationic protein (ECP) in nasal secretions was also measured.
- 2 Intranasal aerosol administration of PAF, 30 or 60 µg per nostril to normal human subjects induced an increased responsiveness to inhaled histamine, 50 to 400 µg and bradykinin, 100 µg per nostril at 2, 6 and 24 h following PAF treatment. However the effect was not apparent at 48 h or 7 days after PAF administration.
- 3 Intranasal administration of lyso-PAF, 60 µg by aerosol did not increase the reactivity of the nasal airway in response to histamine, 200 µg.
- 4 There was no difference in the time course of the PAF-induced hyperresponsiveness to histamine or bradykinin.
- 5 PAF-induced nasal hyperresponsiveness at 2 and 6 h was associated with increases in the ECP concentration of the nasal lavage fluid.
- 6 Vitamin E pretreatment of subjects resulted in the attenuation of the PAF-induced hyperresponsiveness to histamine, and a decrease in ECP levels of the nasal lavage fluid.
- 7 The results suggest that in the human nasal airway, PAF induces a non-specific hyperresponsiveness which is accompanied by eosinophil activation in the nasal cavity. Free radical production induced by PAF may contribute to the hyperresponsiveness and the activation of eosinophils.

Keywords: Platelet-activating factor; human nasal airway; hyperresponsiveness; histamine; bradykinin; vitamin E; eosinophil cationic protein

Introduction

Platelet-activating factor or PAF (1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine), is a membrane-derived phospholipid that has been shown to have a wide variety of biological activities. It increases vascular permeability, is chemoattractant for neutrophils and eosinophils, activates these cells and it induces superoxide anion production and leukotriene synthesis (O'Flaherty & Wykle, 1983). In the lower airways, PAF has been implicated in the pathogenesis of asthma. It has been shown to cause immediate bronchoconstriction (Chung *et al.*, 1986; Cuss *et al.*, 1986) as well as inducing a non-specific bronchial hyperresponsiveness in both animals (Patterson *et al.*, 1984; Chung *et al.*, 1986; Robertson *et al.*, 1988) and man (Cuss *et al.*, 1986).

The mechanism of this hyperresponsiveness is not known. Previous studies have indicated that inflammation is an important factor and that the hyperresponsiveness may involve the release of cytotoxic mediators from eosinophils, such as eosinophil cationic protein (Frigas & Gleich, 1986). However, more recently Webber *et al.* (1992) have provided evidence for PAF-induced hyperresponsiveness *in vitro*, predominantly in the absence of circulating inflammatory cells. This hyperresponsiveness was suggested to be produced by the receptor-mediated release of oxygen free radicals.

Hyperresponsiveness is also seen in the upper airway in patients with allergic rhinitis (Andersson *et al.*, 1987). In order to determine whether PAF might be a potential mediator of hyperresponsiveness in the upper airway, we have studied the effect of inhaled PAF on responsiveness of the human nasal airway in normal subjects towards histamine and bradykinin. We have also investigated, by employing

vitamin E as an oxygen free radical scavenger, the possibility that oxygen free radicals may mediate PAF-induced changes in nasal airway reactivity.

Methods

Subjects

The study was approved by the local Ethics Committee at University College London and all subjects gave their informed consent. For all experiments, normal, healthy volunteers with an age range of 21 to 40 years were used. Subjects suffering from a cold or reporting nasal symptoms were excluded and subjects were taking no medication at the time of, or in the 2 weeks prior to, an experiment. Experiments were performed in conditions kept as constant as possible with respect to temperature and humidity.

Nasal airway resistance measurements

Nasal airways resistance (NAR) was measured by active posterior rhinomanometry using a Mercury Electronics (Glasgow, UK) NR6 Rhinomanometer. The instrument was programmed to calculate nasal resistance at a reference pressure of 75 Pa. The subject held a mask, with airtight seal, over the nose and mouth and breathed through the nose. Airflow was monitored by a pneumotachograph, and pressure within the oropharynx was monitored by an oral pressure cannula placed over the tongue and held through sealed lips.

The instrument was programmed to give the mean NAR over four nasal cycles, and for each subject, three consecutive series of breaths were used to calculate an overall mean of 12

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breaths. Readings were only accepted if the coefficient of variation of the overall mean NAR for a subject was less than 20%.

Study design

In each experiment, subjects were first challenged with saline, then three measurements each of four nasal cycles were taken every 5 min by active posterior rhinomanometry (NAR) over a 15 min period to provide the baseline. To begin with, subjects were challenged with either four doses of histamine (50, 100, 200 and 400 μg) or bradykinin, 100 μg to each nostril. This dose of bradykinin had previously been shown to produce a significant decrease in nasal airway conductance (Proud *et al.*, 1988). After each dose, NAR was measured at 2, 5, 10, 15 and 20 min. Subjects were then treated with vehicle or PAF, 30 or 60 μg to each nostril and subsequent histamine or bradykinin challenges were performed at 2, 6, 24, 48 h and 7 days later. Subjects were allocated to PAF or vehicle treatment groups on a randomized basis. At least 1 week later, subjects were given vehicle or PAF: vehicle if they had previously had PAF and PAF if they had previously had vehicle. Histamine or bradykinin challenges were performed again at 2, 6, 24, 48 h and 7 days after vehicle or PAF administration. In addition, subjects underwent a nasal lavage on each challenge occasion.

In the study with vitamin E, 5 subjects received orally, vitamin E, 400 u daily for 14 days. A blood sample was taken from each subject immediately prior to starting the vitamin E treatment, and another sample was taken on day 14. At this time subjects were treated with PAF, 60 μg to each nostril, as before, and histamine challenges were performed at 2, 6 and 24 h after PAF administration. Nasal lavages were also performed on these occasions.

Nasal lavage

Nasal lavages were performed by a previously described method (Naclerio *et al.*, 1983). Subjects were in a sitting position with the head extended 30° from the horizontal. Warmed sterile saline (5 ml) was instilled in the nostril while the subject abstained from breathing or swallowing. After

10 s the subject leaned forward and expelled the lavage fluid into a collection vessel, which was stored on ice until the completion of the experiment. Approximately 4 ml of the wash was recovered on each occasion. In each experiment, three initial washes were collected in order to remove pre-existing nasal secretions and the third of these was retained and served as a baseline.

The lavage fluid was centrifuged at 4°C for 10 min at 1000 g, after which the supernatants were separated, and stored at -20°C until analysis.

Figure 2 Dose-response curve for histamine and increased nasal airway resistance (NAR) 6 h after pretreatment. (○) Vehicle pretreatment; (●) PAF, 60 μg pretreatment. Changes in NAR are expressed as a percentage of the baseline value which was $0.381 \pm 0.021 \text{ Pa.s.cm}^{-3}$. The data are the means with s.e.mean from 5 separate experiments. *Significant difference ($P < 0.05$) by paired *t* test between PAF and vehicle administration.

Figure 1 Responses to histamine (200 μg), expressed as maximal percentage increase in nasal airway resistance (NAR) above baseline in the post-challenge period, pre-PAF (0 h) and at 2, 6 and 24 h following vehicle or treatment with PAF, 60 μg . (○) Vehicle pretreatment; (●) PAF pretreatment. The baseline NAR was $0.317 \pm 0.016 \text{ Pa.s.cm}^{-3}$. The data are the means with s.e.mean from 5 separate experiments. *Significant difference ($P < 0.05$) by paired *t* test between PAF and vehicle pretreatment.

Figure 3 Responses to bradykinin (100 μg) expressed as maximal percentage increase in nasal airway resistance (NAR) above baseline in post-challenge period, pre-PAF (0 h) and at 2, 6 and 24 h following vehicle or PAF administration. (▽) Vehicle treatment; (●) treatment with PAF, 60 μg . The baseline NAR was $0.343 \pm 0.013 \text{ Pa.s.cm}^{-3}$. The data are the means with s.e.mean from 5 separate experiments. *Significant difference ($P < 0.05$) by paired *t* test between vehicle and PAF administration.

Vitamin E measurements

Blood (5 ml) was obtained by superficial venepuncture of the antecubital fossa, and left to clot at room temperature. The blood was then centrifuged at 1400 g for 10 min, the serum aspirated off, then stored at -20°C until analysis. Vitamin E was assayed by the Department of Chemical Pathology, University College London. The vitamin E was assayed by measuring its fluorescence in hexane (excitation 290 nm; emission 330 nm). The serum samples were saponified with potassium hydroxide in the presence of ascorbic acid, as an anti-oxidant, and ethanol to reduce the extraction of interfering substances (Taylor *et al.*, 1976).

Nasal challenge

Nasal challenge was undertaken with a nasal pump spray (Perfect-Valois, UK Ltd), delivering 100 μl per activation. The spray was placed in one nostril and activated once, then repeated for the opposite nostril. All solutions were at room temperature, and doses of substances were controlled by the concentration of the solutions. Fresh solutions of all challenge agents were made each day from stock solutions stored at -20°C .

Histamine as the diphosphate salt was dissolved in saline (NaCl 154 mM) to make solutions ranging from 1 mg ml^{-1} to 10 mg ml^{-1} . Bradykinin was dissolved in saline to make a 1 mg ml^{-1} solution. PAF was dispersed in distilled water to make a suspension of 0.6 mg ml^{-1} .

Eosinophil cationic protein (ECP) assay

ECP was measured, as previously described (Venge *et al.*, 1977), by a radioimmunoassay.

Data analysis

Changes in NAR in response to each substance were expressed as the percentage change from the NAR values obtained with vehicle challenge. The response to each substance was expressed as the maximal change in NAR for each dose during the post-challenge period.

All data were expressed as mean $\pm$ s.e.mean. Student's *t* tests for paired or independent samples, as appropriate, were applied when comparing the differences between mean values

obtained with the various treatments. A probability value $P < 0.05$ was considered significant.

Materials

Bradykinin was obtained from Peninsula Laboratories, St Helens, Merseyside. Platelet-activating factor (C_{16}) and the lyso form were obtained from Novabiochem, Nottingham. Histamine diphosphate was obtained from Sigma, Poole, Dorset. Vitamin E was manufactured by Pharmadas Ltd, Greenford, Middlesex. The ECP assay kit was produced by Pharmacia Diagnostics, Uppsala, Sweden. All other substances used were of Analar or similar quality.

Results

Following PAF inhalation, no significant change in NAR was measured in any of the subjects. No response was obtained to vehicle alone. All subjects sneezed and experienced nasal itching and nasal blockage after histamine challenge. However, Figure 1 shows that histamine challenge with 200 μg per nostril prior to PAF administration and at 2, 6 and 24 h following PAF resulted in increases in NAR of 79%, 162%, 215%, and 148% respectively, whereas responses to histamine, 200 μg after vehicle treatment were 79%, 89%, 74% and 91% respectively ($n = 5$).

In a separate experiment, when subjects were challenged with histamine, 200 μg the increase in NAR was $71 \pm 17\%$ above baseline ($0.315 \pm 0.015 \text{ Pa.s.cm}^{-3}$) prior to administration of lyso-PAF and $68 \pm 25\%$ above baseline ($0.326 \pm 0.017 \text{ Pa.s.cm}^{-3}$) 6 h after intranasal administration of lyso-PAF, 60 μg ($n = 5$).

The maximum hyperresponsiveness induced by PAF was observed at 6 h, when the percentage increase in NAR following histamine challenge was significantly larger than that obtained before PAF administration ($P < 0.05$) (Figure 2). Subjects challenged with bradykinin experienced nasal blockage without other symptoms and this too was augmented following PAF treatment. Bradykinin-induced increases in NAR prior to PAF administration and at 2, 6 and 24 h after

Figure 4 Duration of responses to histamine and bradykinin, pre-PAF (0 h) and at 2, 6 and 24 h after administration of PAF, 60 μg . (○) Bradykinin, 100 μg ; (●) histamine, 200 μg . The data are the means with s.e.mean from 5 separate experiments.

Figure 5 The effect of PAF administration on the eosinophil cationic protein (ECP) concentration of nasal lavage fluid taken pre-PAF (0 h) and at 2, 6 and 24 h after administration. (●)-PAF, 60 μg ; (▽)-PAF 60 μg and vitamin E treatment. The data are the means with s.e.mean from 3 separate experiments. *Significant change by paired *t* test in ECP concentration compared with that before PAF administration (0 h). †Significant difference by unpaired *t* test in ECP concentration between the untreated subjects and those treated with vitamin E.

PAF were 27%, 32%, 92% and 28% respectively ($n = 5$), as compared with increases of 27%, 30%, 38% and 31% when subjects received vehicle (Figure 3). There was no difference in the duration of the hyperresponsiveness induced by PAF between subjects challenged with histamine and those challenged with bradykinin (Figure 4).

The ECP concentrations in nasal lavage after PAF administration were significantly increased at 2 h and 6 h when compared with the ECP concentrations in the nasal lavage prior to PAF administration. The maximal increase in ECP concentration of the nasal lavage fluid, which occurred at 6 h after PAF administration, was $8.3 \mu\text{g l}^{-1}$ compared with $2.9 \mu\text{g l}^{-1}$ before PAF treatment (Figure 5). Nasal blockage following histamine challenge at 48 h and 7 days after PAF treatment was not significantly different from that without PAF administration (Figure 6).

The effect of the PAF dose required to produce this hyperresponsiveness was investigated and it appeared that the hyperresponsiveness was dose-related (Figure 7).

To study the effect of vitamin E on the PAF-induced hyperresponsiveness, subjects were pretreated with vitamin E 400 u for 14 days. In all subjects, serum levels increased significantly from a mean of $13.2 \pm 1.02 \text{ mg l}^{-1}$ to a mean of $18.2 \pm 1.44 \text{ mg l}^{-1}$; a mean increase of $37.4 \pm 5.2\%$ ($P < 0.01$). On day 14, the PAF-induced hyperresponsiveness to histamine was inhibited (Figure 8), and there was also a corresponding reduction in ECP levels of nasal lavage fluid from $8.3 \mu\text{g l}^{-1}$ without vitamin E pretreatment, to $5.3 \mu\text{g l}^{-1}$ after vitamin E but this was only significant at the 6 h time point (Figure 5).

Discussion

In this paper, we have demonstrated a non-specific increase in responsiveness induced by PAF in the human nasal airway. This hyperresponsiveness is non-specific, as demonstrated by the increase in responses to both histamine and bradykinin following PAF administration. It occurred between 2 and 24 h after PAF administration and was maximal at 6 h. The dose of PAF was chosen from pilot experiments which we conducted and also on the basis of the studies by Andersson & Pipkorn (1988). The effects of the doses we

have studied in the nasal airway are consistent with effects of PAF reported in studies on the human lower airway (Cuss *et al.*, 1986), in primates (Patterson *et al.*, 1984), dogs (Chung *et al.*, 1986) and guinea-pigs (Chapman *et al.*, 1991). However, the precise mechanism of PAF-induced airway hyperresponsiveness is unknown. A variety of mechanisms have been suggested. The PAF-induced hyperresponsiveness has been shown to be dependent on platelet activation (Chapman *et al.*, 1985; Coyle *et al.*, 1990), and is thought to be linked to mediator release from inflammatory cells, particularly eosinophils (Frigas & Gleich, 1986; Sanjar *et al.*, 1990). So in addition to the NAR measurements, we also investigated the possibility of eosinophil activation in this study.

We have shown increases in ECP levels of nasal lavage fluid which have a similar time course to the PAF-induced hyperresponsiveness. This suggests that activation of eosinophils with subsequent release of ECP may contribute to the development of hyperresponsiveness. The interest in examining eosinophil activation in these experiments arose from suggestions that airway hyperresponsiveness may, at least in part, be due to a change in the barrier function of the epithelium (Hogg, 1981), which may be brought about by the release of cytotoxic constituents of eosinophils. A previous study indicated a relationship between ECP levels in nasal secretions and allergen-induced nasal hyperresponsiveness (Linder *et al.*, 1987). However, others (Andersson *et al.*, 1989) found no clear-cut relationship between ECP levels and the induction of hyperresponsiveness. It seems, therefore, that eosinophil recruitment and activation might play a role in nasal hyperresponsiveness, but it is unlikely that it is solely responsible.

Recently, evidence of PAF-induced hyperresponsiveness occurring *in vitro*, largely in the absence of inflammatory cells, has been reported. This is not affected by indomethacin, FPL55712 or mepyramine and cimetidine, but is prevented by catalase, superoxide dismutase (SOD) and WEB2086 (Webber *et al.*, 1992). It has been suggested that a receptor-mediated release of oxygen free radicals may be responsible for this effect, since SOD is a potent scavenger for O_2^- . The protective effect of SOD in other pathological conditions has previously been demonstrated (Rubanyi & Vanhoutte, 1986). In the airway, several reports suggest that oxygen free radicals may be linked to the development of non-specific airway hyperresponsiveness: ozone inhalation can induce hyperresponsiveness in animals (Murlas & Roum, 1985) and

Figure 6 Dose-response curve for histamine and increased nasal airway resistance (NAR) pre-PAF (0 h) and at 24, 48 h and 7 days after PAF, 30 μg . (○) Pre-PAF (0 h); (●) 24 h after PAF; (▽) 48 h after PAF; (▼) 7 days after PAF. The baseline NAR was $0.339 \pm 0.016 \text{ Pa.s.cm}^{-3}$. The data are the means with s.e.mean from 6 separate experiments and bars representing the s.e.mean having been omitted for clarity.

Figure 7 Dose-response curve for histamine and increased nasal airway resistance (NAR) at 24 h after PAF administration. (○) Control; (●) PAF, 30 μg ; (▽) PAF, 60 μg . The baseline NAR was $0.301 \pm 0.014 \text{ Pa.s.cm}^{-3}$. The data are the means from 6 separate experiments and the vertical bars represent the s.e.mean.

Figure 8 Responses to histamine, 200 μ g, pre-PAF (0 h) and at 2, 6 and 24 h after PAF, 60 μ g administration, expressed as the maximal percentage increase in nasal airway resistance (NAR) above baseline during period after challenge with histamine. (○) Control group; (●) vitamin E-treated group. The baseline NAR was 0.295 ± 0.022 Pa.s.cm⁻³. The data are the means with s.e.mean from 5 separate experiments. Significant differences by paired *t* test (**P* < 0.05 and ***P* < 0.01) between no treatment and vitamin E treatment.

man (Seltzer *et al.*, 1986); inhalation of xanthine and xanthine oxidase (an oxygen free radical generating system) has been reported to induce airway hyperresponsiveness in anaesthetized cats (Katsumata *et al.*, 1990); hydrogen peroxide can induce potentiation of contractile responses in isolated rat airway which can be inhibited by catalase (Szarek & Schmidt,

1990), and xanthine oxidase activation is associated with respiratory viral infection (Akaike *et al.*, 1990). We have attempted to examine the role of free radicals in PAF-induced hyperresponsiveness in the nasal airway by use of vitamin E as a free radical scavenger. We have shown that treatment with vitamin E inhibits the PAF-induced increase in responsiveness to histamine and also the accompanying increase in ECP. The dose of vitamin E used has previously been shown to produce a maximum increase in serum levels (Szczeklik, 1987). Vitamin E is a well-documented, effective antioxidant; protecting unsaturated lipids against peroxidation and free radical formation. There is evidence that vitamin E has a similar protective effect in tissues (McCay *et al.*, 1972). It acts by stabilizing and reacting with free radicals to prevent the chain of events by which they lead to cell damage (Fragata & Bellemare, 1980). Assuming that the levels of vitamin E we have produced in the subjects are blocking only free radical formation, then our data suggest that PAF-induced free radical formation is responsible for both hyperresponsiveness and also eosinophil activation. As mentioned above, it is possible that hyperresponsiveness can occur independently of eosinophil activation and support for this comes from experiments showing that hyperresponsiveness occurs *in vitro* where eosinophils are unlikely to be present (Webber *et al.*, 1992). Furthermore, pulmonary eosinophilia has been demonstrated in guinea-pigs, without attendant hyperresponsiveness (Chapman *et al.*, 1991) and *vice versa* (Spina *et al.*, 1991; Herd *et al.*, 1992). However, we cannot exclude the possibility that eosinophil activation acts in concert with other, as yet unknown, mechanisms to produce hyperresponsiveness in the human nasal airway.

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References

- AKAIKE, T., ANDO, M., ODA, T., DOI, T., IJIRI, S., ARAKI, S. & MAEDA, H. (1990). Dependence on O₂⁻ generation by xanthine oxidase of pathogenesis of influenza virus infection in mice. *J. Clin. Invest.*, **85**, 739–745.
- ANDERSSON, M., ANDERSSON, P., VENGE, P. & PIPKORN, U. (1989). Eosinophils and eosinophil cationic protein in nasal lavages in allergen-induced hyperresponsiveness: effects of topical glucocorticosteroid treatment. *Allergy*, **44**, 342–348.
- ANDERSSON, M. & PIPKORN, U. (1988). The effect of platelet activating factor on nasal hypersensitivity. *Eur. J. Clin. Pharmacol.*, **35**, 231–235.
- ANDERSSON, M., VON KOGERER, B., ANDERSSON, P. & PIPKORN, U. (1987). Allergen-induced nasal hyperreactivity appears unrelated to the size of the nasal and dermal immediate allergic reaction. *Allergy*, **42**, 631–637.
- CHAPMAN, I.D., BOUBEKEUR, K. & MORLEY, J. (1991). Airway eosinophilia and airway hyperreactivity are parallel rather than sequential events in the guinea-pig. *Agents Actions*, **34**, 445–456.
- CHUNG, K.F., AIZCWA, H., LEIKAUF, G.D., UEKI, I.F., EVANS, T.W. & NADEL, J.A. (1986). Airway hyperresponsiveness induced by platelet-activating factor: role of thromboxane generation. *J. Pharmacol. Exp. Ther.*, **236**, 580–584.
- COYLE, A.J., SPINA, D. & PAGE, C.P. (1990). PAF-induced bronchial hyperresponsiveness in the rabbit: contribution of platelets and airway smooth muscle. *Br. J. Pharmacol.*, **101**, 31–38.
- CUSS, F.M., DIXON, C.M.S. & BARNES, P.J. (1986). Effects of inhaled platelet-activating factor on pulmonary function and bronchial responsiveness in man. *Lancet*, **ii**, 189–192.
- FRAGATA, M. & BELLEMARE, F. (1980). Model of singlet oxygen scavenging by α -tocopherol in biomembranes. *Chem. Phys. Lipids*, **27**, 93–99.
- FRIGAS, E. & GLEICH, F.J. (1986). The eosinophil and the pathophysiology of asthma. *J. Allergy Clin. Immunol.*, **77**, 527–537.
- HOGG, J.C. (1981). Bronchial mucosal permeability and its relationship to airway hyperreactivity. *J. Allergy Clin. Immunol.*, **67**, 421–425.
- HERD, C.M., DONIGI-GALE, D., SHOUPZ, T.S. & PAGE, C.P. (1992). Effect of a combined 5-lipoxygenase inhibitor and leukotriene antagonist (PF 5901) on PAF-induced airway responsiveness and cellular infiltration in neonatally immunised rabbit. *Br. J. Pharmacol.*, **107**, 1108–1115.
- KATSUMATA, U., MIURA, M., ICHINOSE, M., KIMURA, K., TAKAHASHI, Y., INOUE, H. & TAKISHIMA, T. (1990). Oxygen radicals produce airway constriction and hyperresponsiveness in anaesthetized cats. *Am. Rev. Respir. Dis.*, **141**, 1158–1161.
- LINDER, A., VENGE, P. & DEUSCHL, H. (1987). Eosinophil cationic protein and myeloperoxidase in nasal secretions as markers of inflammation in allergic rhinitis. *Allergy*, **42**, 583–590.
- MCCAY, P.B., PFEIFER, P.M. & STIPE, W.H. (1972). Vitamin E protection of membrane lipids during electron transport functions. *Ann. N. Y. Acad. Sci.*, **203**, 62–73.
- MURLAS, C.G. & ROUM, J.H. (1985). Sequence of pathologic changes in the airway mucosa of guinea pigs during ozone-induced bronchial hyperreactivity. *Am. Rev. Respir. Dis.*, **131**, 314–320.
- NACLERIO, R.M., MEIER, H.L., KAGEY-SOBOTKA, A., ADKINSON, N.F., MEYERS, D.A., NORMAN, P.S. & LICHTENSTEIN, L.M. (1983). Mediator release after nasal airway challenge with allergen. *Am. Rev. Respir. Dis.*, **128**, 597–602.
- O'FLAHERTY, J.T. & WYLLIE, R.L. (1983). Biology and biochemistry of platelet activating factor. *Clin. Rev. Allergy*, **1**, 353–367.

- PATTERSON, R., BERNSTEIN, P.R., HARRIS, K.E. & KRELL, R.D. (1984). Airway responses to sequential challenge with platelet-activating factor and leukotriene D₄ in rhesus monkeys. *J. Lab. Clin. Med.*, **104**, 340–345.
- PROUD, D., REYNOLDS, C.J., LACAPRA, S., KAGEY-SOBOTKA, A., LICHTENSTEIN, J.M. & NACLERIO, R.M. (1988). Nasal provocation with bradykinin induces symptoms of rhinitis and a sore throat. *Am. Rev. Respir. Dis.*, **137**, 613–616.
- ROBERTSON, D.N., COYLE, A.J., RHODEN, K.J., GRANDORDY, B., PAGE, C.P. & BARNES, P.J. (1988). The effect of platelet activating factor on histamine and muscarinic receptor function in guinea-pig airways. *Am. Rev. Respir. Dis.*, **137**, 1317–1322.
- RUBANYI, G.M. & VANHOUTTE, P.M. (1986). Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. *Am. J. Physiol.*, **250**, H822–827.
- SANJAR, J.L., AOKI, S., BOUBEKEUR, K., CHAPMAN, I.D., SMITH, D., KINGS, M.A. & MORLEY, J. (1990). Eosinophil accumulation in pulmonary airways of guinea-pigs induced by exposure to an aerosol of platelet-activating factor: effect of anti-asthma drugs. *Br. J. Pharmacol.*, **99**, 267–272.
- SELTZER, J., BIGBY, B.G., STULBARG, M., HOLTZMAN, M.J., NADEL, J.A., UEKI, I.F., LEIKAUF, G.D. & GOETZL, E.J. (1986). O₃-induced change in bronchial reactivity to methacholine and airway inflammation in humans. *J. Appl. Physiol.*, **60**, 1321–1326.
- SPINA, D., MCKENNIFF, M.G., COYLE, A.J., SEEDS, E.A.M., TRAMONTANA, M., PERRETTI, F., MANZINI, S. & PAGE, C.P. (1991). Effect of capsaicin on PAF-induced bronchial hyperresponsiveness and pulmonary cell accumulation in the rabbit. *Br. J. Pharmacol.*, **103**, 1268–1274.
- SZAREK, J.L. & SCHMIDT, N. (1990). Hydrogen peroxide-induced potentiation of contractile responses in isolated rat airways. *Am. J. Physiol.*, **258**, L232–L237.
- SZCZEKLIK, A. (1987). Antioxidant effect of vitamin E in hyperlipoproteinemias. *Agents Actions*, **22**, 359.
- TAYLOR, S.C., LAMDEN, M.P. & TAPPEL, A.L. (1976). Sensitive fluorimetric method for tissue tocopherol analysis. *Lipids*, **11**, 530–538.
- VENGE, P., ROXIN, L.E. & OLSSON, I. (1977). Radioimmunoassay of human eosinophil cationic protein. *Br. J. Haematol.*, **37**, 331–335.
- WEBBER, S.E., MORIKAWA, T. & WIDDICOMBE, J.G. (1992). PAF-induced muscarinic cholinergic hyperresponsiveness of ferret tracheal smooth muscle and gland secretion *in vitro*. *Br. J. Pharmacol.*, **105**, 230–237.

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