

Study of the effects of paf-acether on human nasal airways

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In 6 normal subjects and 6 patients with allergic rhinitis, nasal response to insufflation of paf-acether (paf, platelet-activating factor), lyso-paf and histamine was evaluated. Nasal challenge with paf, at doses of 300 and 600 nM, induced nasal obstruction, associated with an increase in nasal airway resistances, measured by anterior passive rhinomanometry. Maximum increase in nasal airway resistance was observed at 30 min after challenge (mean percent change + 481 with 600 nM paf; $P < 0.05$). Other symptoms induced by paf insufflation were rhinorrhea (6 out of 12 subjects), itching (8 out of 12), sneezing (4 out of 12) and a burning sensation (6 out of 12). No differences were observed between normal and rhinitic subjects, concerning nasal sensitivity to paf. Neither nasal symptoms nor changes in nasal airway resistance were observed after nasal challenge with lyso-paf (300 and 600 nM); by contrast, histamine (100 nM) induced sneezing, nasal obstruction, itching and rhinorrhea in all the studied subjects, associated with an increase in nasal airway resistance (maximum 5 min after challenge; percent change + 358; $P < 0.02$). Nasal effects of paf were not mediated by histamine, since no increase in histamine levels was observed in nasal washings following paf insufflation. We conclude that paf may have pathogenetic relevance in allergic rhinitis.

Key words: allergic rhinitis; nasal airway resistance; nasal mucosa; paf-acether.

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Several data suggest that paf-acether (paf, platelet-activating factor) is an important mediator of allergic and inflammatory reactions. This phospholipid is endowed with a wide range of biological activities, including bronchoconstrictor properties and the capability of inducing bronchial hyperresponsiveness (4, 7, 21). Moreover, paf was shown to be a potent chemotactic and activating factor for human eosinophils (12, 13, 27) and human neutrophils (22). Both cell populations are supposed to be involved in the pathogenesis of allergic inflammation, particularly of the late antigen-induced reactions (6, 8, 10). Recently, it was demonstrated that paf and its precursor/metab-

olite lyso-paf are released in nasal secretions after local antigen stimulation in patients with hay-fever (14, 16). Moreover, it has been shown that early and late phase antigen-induced reactions in the nose are associated with an increase in eosinophils and neutrophils in nasal washings (3, 19). These data suggest that paf may be involved in the pathogenesis of allergic reactions in upper respiratory airways.

To investigate the possible role of paf in allergic rhinitis, we evaluated nasal symptoms and nasal airway resistance (NAR) after local stimulation with paf, lyso-paf and histamine in normal subjects and in hay-fever patients.

MATERIAL AND METHODS

Subjects

Six healthy subjects and 6 patients affected with allergic rhinitis, with ages ranging from 18 to 34 years (mean 24 years) were recruited for the study. The rhinitic patients were allergic to grass pollens, as indicated by clinical story and skin prick tests. The study was carried out in single blind, that is to say that the subjects did not know what was insufflated in the nose. Nasal provocations were performed outside the grass pollen season, when the patients were symptom free and were not taking any medication. All the subjects gave their informed consent prior to study.

Nasal challenge

Paf (1-0-hexadecyl-2-acetyl-sn-glycero-3-phosphorylcholine, purchased from Bachem, Bubendorf, Switzerland) and lyso-paf (1-0-hexadecyl-sn-glycero-3-phosphorylcholine, Bachem) were dissolved in 100 % ethanol (final concentration 1 %) and diluted in saline solution (0.9 % w/v NaCl) containing 0.25 % human serum albumin. Paf and lyso-paf solutions were aliquoted in small amounts and frozen at -20°C. The solution employed was thawed just before nasal challenge. The biological activity of paf solution was evaluated at regular intervals by intradermal injection in volunteers. Histamine dihydrochloride (Carlo Erba, Milan, Italy) was diluted in saline solution. Nasal provocations with the paf diluent were performed as control. Paf, lyso-paf and histamine were insufflated in both nostrils by means of a nasal pump spray, delivering a volume of 150 µl. Before challenge, the subjects waited 30 min to allow the nasal mucosa to become acclimatized. Nasal airway resistance was measured by an anterior passive rhinomanometer (Markos, Monza, Italy). This delivered an airstream with a fixed flow of

0.25 l/s into each nostril. The resistances for the left (R_L) and the right (R_R) nostrils were calculated by dividing the nasal pressure by the flow, and were expressed in Pascal/cm³/sec). The total nasal resistance (R_{TOT}) was calculated by the following formula:

$$R_{TOT} = \frac{R_L \times R_R}{(R_L + R_R)}$$

Mean percent changes in NAR are reported in the results. They have been calculated as follows: [(Postchallenge R_{TOT} - Prechallenge R_{TOT})/Prechallenge R_{TOT}] × 100.

Measurements were carried out before and 5, 10, 20, 30 and 60 min after nasal challenge with different doses of paf (total amount administered ranged from 60 to 600 nM), lyso paf (300 and 600 nM) and histamine (100 nM). Mean values of triplicate measurements were considered.

Symptom registration

The subjects were asked to record the severity of rhinorrhea, nasal obstruction, itching, sneezing and burning sensation after provocation with paf, histamine and the paf diluent. Scores for each symptom ranged from 0 to 4 (none = 0, mild = 1, moderate = 2, intense = 3, very intense = 4). Sneezes were classified according to the number (0 = 0, 1 to 5 sneezes = 1, 6 to 10 sneezes = 2, 11 to 15 sneezes = 3, more than 15 sneezes = 4).

Histamine determination in nasal secretions after challenge with paf

To determine whether the effect of paf on nasal airways is direct or mediated by histamine release from nasal mucosa mast cells, histamine concentrations in nasal secretions were measured before and after challenge with paf in 10 subjects (5 allergic and 5 nonallergic). Nasal lavage fluids were collected using the technique of Naclerio et al. (18), with minor modifications (15). Briefly, 5 ml of saline solution (0.9 % NaCl) were instilled into each nostril. After 10 s, the subject expelled

the mixture of mucus and saline solution, which was collected into conical polypropylene tubes (Falcon, Becton Dickinson, Oxnard, CA, USA). The nasal lavage fluid was centrifuged at 4°C for 15 min at 15,000 *g*, the supernatant was aspirated and stored at -20°C until assayed for histamine concentration. Five successive nasal washes were performed before challenge with paf. Subsequently, nasal lavages were repeated 5, 10, 20, 30 and 60 min after provocation.

Histamine determination

Supernatants from nasal lavage fluids were diluted $\frac{1}{2}$ with 4% v/v HClO₄, centrifuged at 4°C for 15 min at 2,000 *g*, and assayed in duplicate for histamine concentration by an automated fluorometric technique (23).

Statistical analysis

Results were expressed as mean $\pm$ SEM. Statistical analysis was performed by two tailed Student's *t* test for paired data (nasal airway resistances at different postchallenge times were compared with prechallenge values).

RESULTS

Symptom scores and nasal airway resistance

Nasal challenge with histamine (100 nM) induced rhinorrhea, itching, sneezing and

nasal obstruction, associated with a statistically significant increase in NAR in all the study subjects, without any difference between rhinitic patients and normal subjects. Time courses of symptom scores and NAR show a peak 5 min after challenge and a progressive decrease in the first hour (Figs. 1 and 2).

Nasal provocation with 60 nM paf did not induce either relevant symptoms or modifications in NAR in any of the studied patients. Conversely, nasal symptoms were observed after challenge with 300 and 600 nM paf. The most relevant symptom was nasal obstruction (in 10 of 12 subjects); other symptoms were rhinorrhea (6 of 12), itching (8 of 12), sneezing (4 of 12) and a burning sensation (6 out of 12) (Figs. 3a, 3b, 3c). NAR were significantly increased with 300 and 600 nM at 10, 20 and 30 min after challenge, with a maximum between 20 and 30 min (Fig. 4). A progressive decrease in NAR was observed after the first 30 min. No difference was observed between normal and rhinitic subjects, concerning nasal sensitivity to paf.

Nasal provocation with lyso-paf (300 and 600 nM) or paf diluent neither induced any symptoms nor modified NAR.

Histamine determination in nasal secretions after challenge with paf

Nasal challenge with 600 nM paf was not followed by any appreciable histamine release

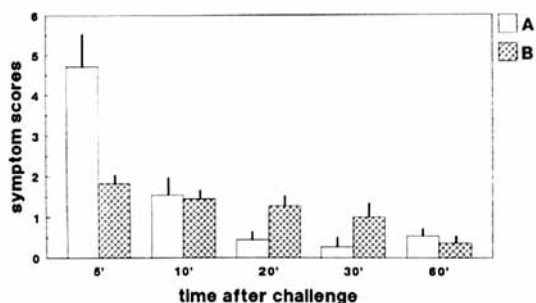


Fig. 1. Time course of symptom scores after nasal challenge with 100 nM histamine in 12 subjects (6 normals and 6 with allergic rhinitis). A = rhinorrhea, itching, sneezing; B = nasal obstruction. Data are expressed as mean $\pm$ SEM.

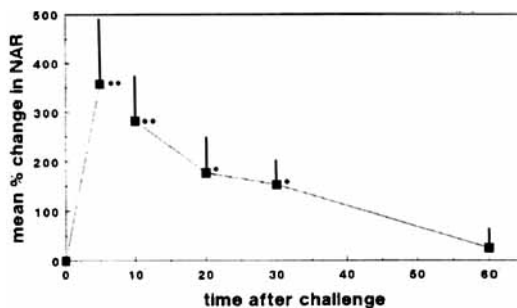


Fig. 2. Mean percent changes of nasal airway resistance (NAR) after nasal challenge with 100 nM histamine in 12 subjects. NAR were measured by anterior passive rhinomanometry and calculated as Pascal/cm³/s. **P* < 0.05, ***P* < 0.02.

in nasal secretions in any of the studied subjects. As described elsewhere (15), high histamine levels were detected in the first pre-challenge nasal lavages. With repeated lavages, a progressive decrease in histamine levels was observed until low and uniform levels were obtained at the fifth prechallenge lavages. After nasal challenge with paf, no modifications of histamine levels were found (Fig. 5).

DISCUSSION

Nasal challenge with paf induces a dose-dependent and reversible increase in NAR, asso-

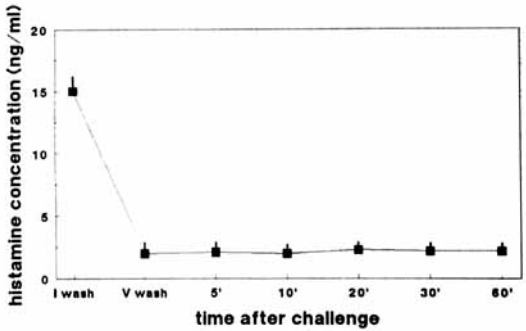


Fig. 4. Mean percent changes of nasal airway resistances after nasal challenge with the doses of paf shown, in 12 subjects. Nasal airway resistances were measured by anterior passive rhinomanometry and calculated as Pascal/cm³/sec **P* < 0.05, ***P* < 0.02.

ciated with nasal symptoms, such as nasal obstruction, itching, rhinorrhea, sneezing and a burning sensation. Nasal obstruction was the most relevant and lasting symptom; itching, rhinorrhea and sneezing were experienced only by some subjects and were usually mild. Time course of NAR demonstrates that paf exerts a maximum of activity at 20–30 min after challenge. Normal and rhinitic subjects share the same sensitivity to nasal provocation with paf.

The effects of paf on the nose could be the result of the release of a secondary mediator, since it was demonstrated that this lipid mediator can induce histamine release from rat kidney (1) and leukotriene generation from lung tissue (26). To determine whether paf effects on the nose are mediated by histamine

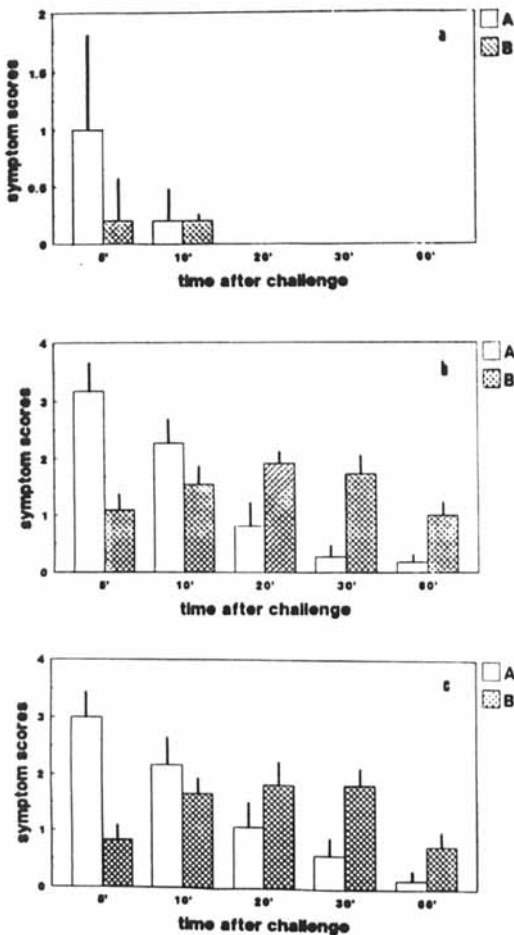


Fig. 3a, 3b and 3c. Time courses of symptom scores after nasal challenge with different doses of paf (3a: 60 nM paf; 3b: 300 nM paf; 3c: 600 nM paf). A = rhinorrhea, itching, sneezing, burning sensation; B = nasal obstruction.

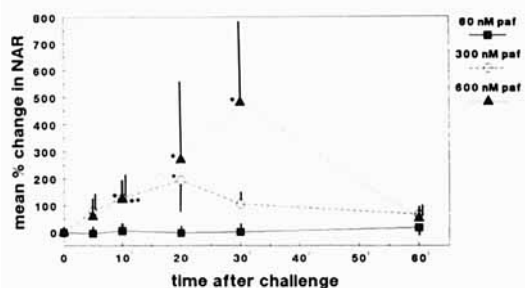


Fig. 5. Time course of histamine levels in nasal lavage fluids after nasal challenge with 600 nM paf in 10 subjects.

release from nasal mucosa mast cells, we evaluated histamine release in nasal secretions after provocation with paf. No changes in histamine levels were observed, and therefore we concluded that the effects in the nose are not mediated by histamine. This is also confirmed by the different symptom pattern and the different time course of the symptoms elicited by nasal challenge with histamine. Nasal stimulation with histamine induced itching, sneezing, rhinorrhea, nasal obstruction and an increase in NAR, peaking 5 min after challenge. When compared to the effects of paf, itching, sneezing and rhinorrhea were more pronounced, whereas nasal obstruction was milder and lasted for a shorter period.

Paf has a wide range of biological activities, such as increased vascular permeability (9), bronchial smooth muscle contraction (24), chemoattraction of eosinophils and neutrophils (27) and activation of several cell populations, as eosinophils (13), neutrophils (22), platelets (5), macrophages (11) and monocytes (25). Moreover, paf can induce nasal and bronchial hyperresponsiveness to antigenic stimuli (2, 7). The increase in NAR and the nasal obstruction induced by paf reflect a thickening of the nasal mucosa. This could be related to an increase in volume of the capacitance vessels, an increase in interstitial fluid, and/or mucosal infiltration by inflammatory cells, as neutrophils and eosinophils. The effects of paf on human nasal airways could be, at least in part, reflex-mediated, since rhinorrhea and sneezing were observed respectively in 6 and in 4 subjects out of 12. Sneezing is mediated through reflex activity, and nasal secretion may be reflex-mediated or related to a direct extravasation of plasma from the vasculature. It is noteworthy that 6 subjects of 12 experienced a burning sensation after challenge with paf. This suggests that paf may stimulate pain receptors in the nasal mucosa.

Our results are at variance with those of previous investigators. Pipkorn et al. (20) found that nasal provocation with paf did not cause any significant symptom; by contrast, it induced a slight decrease in NAR. However, when we utilized the same dose of paf employ-

ed by Pipkorn et al. (about 50 nM), we did not observe any relevant symptom; the increase in NAR and the nasal symptoms were in fact obtained only with higher doses of paf (300 and 600 nM). These doses are higher than those found in nasal washings after local antigen challenge in rhinitic patients (16). However, the relation between the amount of paf generated and that found in nasal secretions is not known. It has been demonstrated that more than 95% of exogenously administered paf binds quickly to its putative receptors in respiratory airways of experimental animals (17). It is reasonable to suppose that the amount of paf found in nasal washings is much lower than that generated; therefore, the dose of paf employed in our study may have a physiopathological relevance. However, on a molar basis, paf appears to be less potent than histamine in inducing nasal symptoms.

It has been demonstrated that paf metabolic pathway is activated following nasal antigen challenge in hay-fever patients (14, 16). Now we demonstrate that paf can induce an increase in NAR, associated with nasal obstruction and, sometimes, with itching, sneezing, rhinorrhea, and a burning sensation. These results and the observations of Andersson et al. (2) that paf can induce nasal hyperreactivity to a subsequent antigen challenge in rhinitic patients suggest that paf is a potential mediator of allergic rhinitis.

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