Platelet-activating factor (PAF) is a potent inflammatory mediator mainly by activated macrophages and eosinophils, which might play a role in asthma [1]. PAF has been detected in bronchoalveolar lavage (BAL) of stable asthmatics and raised levels of this mediator have been reported in blood of symptomatic or destabilized asthmatics [2–4]. Inhalation of PAF has been shown to induce bronchoconstriction and an increase in airway responsiveness in normal [5, 6] and asthmatic subjects [7]. However, PAF causes a variable contraction of human airways in vitro and does not seem to act directly on airway smooth muscle [8, 9]. The mechanism by which inhaled PAF induces airway obstruction in vivo remains speculative, although some secondary mediators may be involved [10].

Bronchial hyperresponsiveness to several types of stimuli is the functional hallmark of asthma. Studies comparing the acute airway obstruction after PAF inhalation in normal and asthmatic subjects are still sparse and have yielded conflicting results. Rubin et al. [6] assessed the variations in specific airway conductance (SGaw), expiratory flow rate at 30% of vital capacity (Vp30), and forced expiratory volume in one second (FEV1). They found no significant difference between normal and asthmatic groups when performing dose-response curves over a dose range from 2.3 ng to 23 µg, although they reported that only the asthmatics displayed a significant fall in FEV1 after the last dose. Chung and Barnes [11] found a comparable fall in Vp30 in mild asthmatics and normal subjects after inhalation of doses ranging 12–24 µg. By contrast, using both SGaw and forced expiratory flow rates to assess the bronchial response, we have recently demonstrated a clearly more marked acute airway obstruction following inhalation of PAF in asthmatic and normal subjects: comparison with methacholine. R.E. Louis, M.F. Radermecker.

Acute bronchial obstruction following inhalation of PAF in asthmatic and normal subjects: comparison with methacholine. R.E. Louis, M.F. Radermecker. ©ERS Journals Ltd 1996.

ABSTRACT: Platelet-activating factor (PAF) may play a role in the pathophysiology of asthma but controversies exist about bronchial responsiveness toward this mediator in asthma.

We have compared the variations in the specific conductance (SGaw) and forced expiratory volume in one second (FEV1) in 12 asthmatics and 12 normal subjects after inhalation of doubling doses of PAF (15–120 µg) and methacholine (18 to at least 144 µg). In order to take into account a possible tachyphylaxis, we compared PAF dose-response curves performed on one day with the curves obtained by giving the same doses separately on different days.

Repeated inhalations of doubling doses of PAF caused SGaw and FEV1 to plateau after the second dose in each group, whereas methacholine provoked a dose-related decrease in SGaw and FEV1. A dose-dependent decrease in the functional indices was restored when the different doses of PAF were administered on separate days. In both groups, the fall in SGaw after inhalation of 60 µg as a single dose was higher than that achieved when this dose was given during a full bronchial challenge. The falls in SGaw and FEV1 after PAF inhalation were significantly higher in the asthmatics than in the normal subjects. The provocative dose of PAF causing a 35% fall in SGaw (PD35,SGaw) PAF was only twofold lower in the asthmatics than in the normal subjects (p<0.05), while it was 11 fold lower for methacholine (p<0.001). When the PD35,SGaw values were compared, PAF was found on a molar basis to be 33 fold more potent than methacholine in the normal subjects, but only fivefold more potent in the asthmatics (p<0.05) and PAF (p=0.09).

Our results demonstrate a tachyphylaxis after inhalation of platelet-activating factor in normal subjects and asthmatics, and show that asthmatics develop a greater bronchial obstruction than normal subjects even if methacholine is more sensitive than platelet-activating factor at discriminating between the two groups.

Eur Respir J., 1996, 9, 1414–1420.
in asthmatics than in normal subjects after inhalation of a single dose of 30 µg PAF [12]. In a study by HSIEH [13], inhalation of doubling doses of PAF resulted in a greater fall in FEV1 among asthmatic children as compared to normal controls. A tachyphylaxis of the bronchial response after repeated inhalations of increasing doses of PAF has been previously reported by some [5, 14], but not all authors [6, 15, 16], and makes the comparison between the study of RUBIN et al. [6] and our previous data difficult.

Given the controversies about the bronchial responsiveness to inhaled PAF in asthmatics, we have investigated the variations in sGaw and FEV1 generated by inhalation of doubling doses of PAF and methacholine in 12 asthmatic and 12 normal subjects. In order to reveal a possible tachyphylaxis to PAF, the PAF dose-response curves performed on one day were compared with the curves obtained by giving the same doses separately on different days.

**Material and methods**

**Study design**

Twelve normal subjects and 12 atopic asthmatics volunteered for the study. At the first visit, the subjects were screened for their baseline lung function, their bronchial methacholine responsiveness (determination of the provocative concentration producing a 20% fall in FEV1 (PC20,FEV1)) and their sensitivity to common aeroallergens (Dermatophagoides pteronyssinus, grass pollen, tree pollen and moulds) by skin-prick test (table 1). All the asthmatic subjects had a clinical history of asthma [17], and were in a stable condition at the beginning of the study. Each subject underwent four bronchial challenges, 3 weeks apart: a dose-response methacholine challenge (Visit 1); a dose-response PAF challenge (Visit 2); a single dose 30 µg PAF challenge (Visit 3); and a single dose 60 µg PAF challenge (Visit 4). All the bronchial challenges were performed in a single-blind manner, at the same time of the day (0900–1100). For safety reasons, it was decided to conduct the different challenges in a nonrandomized fashion in the order described above. Indeed, we have previously observed a dramatic fall in FEV1 after inhalation of a single dose of 30 µg in some asthmatic patients [12]. Inhaled bronchodilators were stopped at least 12 h before each test and caffeine containing beverages were not consumed within 4 h before the challenges. None of the subjects reported clinical symptoms of upper respiratory tract infection during the study period. The study was approved by our local Ethical Committee and all subjects gave their written informed consent.

| Subject No. | Age yrs | Sex | Tobacco | Atopy | FEV1 % pred | PC20 mg·mL⁻¹ | Drugs |
|-------------|---------|-----|---------|-------|-------------|--------------|-------|
| **Asthmatics** | | | | | | | |
| 1 | 25 | F | No | Yes | 91 | 0.43 | SO+CrR |
| 2 | 26 | M | No | Yes | 102 | 1.20 | SO |
| 3 | 30 | M | No | Yes | 110 | 3.13 | SO |
| 4 | 28 | M | No | Yes | 118 | 0.28 | SO |
| 5 | 30 | F | No | Yes | 103 | 0.17 | SO |
| 6 | 24 | F | No | Yes | 106 | 2.36 | SO |
| 7 | 23 | F | Yes | No | 96 | 0.43 | SO |
| 8 | 25 | F | No | Yes | 83 | 0.09 | SO+CrR |
| 9 | 21 | F | No | Yes | 86 | 0.07 | SO+CrR |
| 10 | 22 | F | No | Yes | 86 | 0.07 | SO |
| 11 | 24 | M | No | Yes | 77 | 0.08 | SO |
| 12 | 23 | F | No | Yes | 94 | 2.3 | SO |
| Mean | 25 | 96 | 0.37* |
| **Normals** | | | | | | | |
| 1 | 36 | M | Yes | No | 108 | >16 | No |
| 2 | 26 | M | No | No | 112 | >16 | No |
| 3 | 28 | F | No | No | 77 | >16 | No |
| 4 | 37 | M | No | No | 93 | >16 | No |
| 5 | 41 | F | Yes | No | 104 | >16 | No |
| 6 | 27 | M | No | Yes | 86 | >16 | No |
| 7 | 21 | M | No | No | 100 | >16 | No |
| 8 | 21 | M | No | No | 101 | >16 | No |
| 9 | 33 | F | Yes | No | 109 | >16 | No |
| 10 | 21 | M | No | No | 89 | >16 | No |
| 11 | 29 | F | No | No | 109 | >16 | No |
| 12 | 20 | F | No | No | 126 | >16 | No |
| Mean | 28 | 101 |

*: geometric mean. F: female; M: male; FEV1: forced expiratory volume in one second; PC20: provocative concentration of methacholine causing a 20% fall in FEV1; SO: salbutamol occasionally; CrR: cromoglycate regularly; ICoR: inhaled corticoids regularly.
Methacholine challenge

Methacholine chloride solutions (Biochemicals) were dissolved in saline solution, stored at 4°C, and used within 2 weeks of preparation. On the screening day, the PC20 methacholine was determined according to the method described by Cockcroft et al. [18], starting with 0.03 and 1 mg·mL−1 in asthmatic and normal subjects, respectively, and reaching maximally 16 mg·mL−1. The aerosols were delivered by a jet nebulizer (Hudson), the characteristics of which have been described previously [12].

On Visit 1 of the protocol, both asthmatic and normal subjects inhaled doubling doses of methacholine every 20 min, starting at 0.03 mg·mL−1 (18 µg aerosolized), up to at least 0.25 mg·mL−1 (144 µg aerosolized). Measurements of sGaw (Plethysmography; Bodytest, Jaeger) and FEV1 (Flow screen; Jaeger) were successively performed 5, 10 and 15 min after each dose, and the lowest value was retained for drawing the dose-response curve. The sGaw was recorded as the mean of three values, whilst FEV1 was recorded as the best of three manoeuvres. If a fall of at least 35% sGaw had not occurred after inhalation of 0.25 mg·mL−1, the test was carried on by doubling the dose until this threshold was reached. The PD35, sGaw was interpolated from the dose-response curve. Any fall in FEV1 higher than 20% was reversed by inhaled salbutamol if the FEV1 had dropped by more than 30%, the subject was kept under medical control for 1 h after the challenge and released afterwards when the FEV1 has returned to within 20% of control.

PAF challenge

A stock solution and appropriate dilutions of PAF (1-0-alkyl-2-0-acetyl-sn-glyceryl-3-phosphorylcholine, Sigma) (25, 50, 100 and 200 µg·mL−1) were prepared as described previously [12] from a vial containing 2 mg·mL−1 solution in chloroform. Each solution contained 2% ethanol, was kept at 4°C and used within 48 h. Both the nebulizer and the procedure of inhalation (2 min, 2% ethanol) were kept at 4°C and used within 48 h. Any fall in sGaw > 20% from control value was reversed by inhaled salbutamol at the end of the test, as described above for methacholine.

Statistical analysis

Results are expressed as mean±SEM unless otherwise indicated. Baseline values of sGaw and FEV1 on the different study days were assessed by two-way analysis of variance (ANOVA). The significance of the decrease in sGaw and FEV1 after inhalation of different doses of PAF or methacholine within each group was assessed by one-way repeated measure ANOVA, followed by a Neumann-Keul test to compare the effects of the different doses together or with control. The comparison of the decrease in sGaw and FEV1 after the different doses between asthmatic and normal subjects was made by using an unpaired t-test. The comparison of the PD35, sGaw PAF or PD35, sGaw methacholine, as well as the comparison of their ratio between asthmatic and normal subjects, were performed using an unpaired t-test on log transformed values. The comparison between the falls in sGaw and FEV1 after 60 µg PAF, either inhaled as a single dose or during a full dose response challenge, was performed using a paired t-test. Correlation between PD35, sGaw PAF and PD35, sGaw methacholine was assessed by calculating the Spearman's coefficient. A p-value equal to or less than 0.05 was considered to be statistically significant.

Results

There was no significant difference in baseline FEV1 or sGaw between the asthmatic and normal subjects on the different study days (two-way ANOVA) (table 2). Successful inhalations of doubling doses of PAF (15–120 µg) caused an airway obstruction in normal and asthmatic subjects which rapidly plateaued (fig. 1a). In asthmatics, airway obstruction, assessed by a decrease both in FEV1 and sGaw, did not rise significantly further after 15 µg (repeated one-way ANOVA) and clearly plateaued after inhalation of 30 µg. In normal subjects, the fall in sGaw did not increase significantly further after 15 µg, and the variations in FEV1 were clinically irrelevant (<5%) at all doses of PAF tested. Significant falls from baseline both in FEV1 (8.3±2.9%; p<0.05) and sGaw (30±5.4%; p<0.001) were already achieved with inhalation of 15 µg PAF in the asthmatic group, while the fall in sGaw became statistically significant after 30 µg inhaled PAF in the normal subjects (25±6.4%; p<0.01). The falls in FEV1 were significantly higher in the asthmatic than in the normal subjects at all doses of PAF tested, while significant differences for the falls in sGaw between the two groups occurred from the dose of 30 µg.

In addition to causing bronchoconstriction, inhaled PAF resulted in a throat irritation in almost all the subjects and in a facial warmth in some of them. This phenomenon
was essentially observed after the first or the second aerosolized dose.

The dose-response curve obtained after inhalation of different single doses of PAF (15–60 µg) performed on separate days did not show any plateau (fig. 1b). The falls in $s_{Gaw}$ after 60 µg PAF were significantly greater than those observed after 15 µg both in asthmatic (p<0.001) and normal subjects (p<0.01); and the fall in FEV1 after 60 µg in asthmatics tended to be greater than that after 15 µg (p=0.06). The falls in FEV1 were significantly more pronounced in asthmatics than in normal subjects for all doses tested. The falls in $s_{Gaw}$ were significantly higher in asthmatics after inhalation of 30 and 60 µg.

Both in asthmatic and normal subjects, the maximal decreases in $s_{Gaw}$ after 60 µg PAF were significantly greater than those recorded when the same dose of PAF was administered during a full bronchial challenge (table 3). Although a similar trend was observed with FEV1 in the asthmatic subjects, the difference was not significant.

Inhalation of similar doubling doses of methacholine (18–144 µg) caused a definite dose-related airway obstruction in asthmatic subjects, with no plateau (fig. 2). The bronchial response in the normal subjects was very weak, and the differences between asthmatic and normal subjects were significant at all doses of methacholine tested both for FEV1 and $s_{Gaw}$.

PD35, $s_{Gaw}$ PAF could be determined in eight subjects of each group during the full bronchial challenge (table 4). The PD35, $s_{Gaw}$ PAF was slightly but significantly lower in the asthmatics (p<0.05). In the same subjects, PD35, $s_{Gaw}$ methacholine was strikingly lower in the asthmatics than in the normals (p<0.001). On a molar basis, PAF (560 molecular weight (MW)) was a more potent bronchoconstrictor agent than methacholine (196 MW) in both groups. However, the relative bronchoconstrictor potency of PAF versus methacholine was significantly higher in normal subjects than in asthmatics.

Table 3. – Comparison between the acute bronchial obstruction caused by 60 µg inhaled PAF given either as a single dose or during a dose response challenge

|                | 60 µg single dose | 60 µg dose response curve* | p-value |
|----------------|-------------------|---------------------------|---------|
| Asthmatics     |                   |                           |         |
| Fall in FEV1   | 17±3              | 14±3                      | >0.05   |
| Fall in $s_{Gaw}$ | 64±5            | 48±6                      | <0.05   |
| Normals        |                   |                           |         |
| Fall in FEV1   | 3.6±0.8           | 3.2±1.4                   | >0.05   |
| Fall in $s_{Gaw}$ | 44±6            | 22±8                      | <0.001  |

* 105 µg cumulative dose. Values are presented as mean±SEM. For abbreviations see legend to table 2.
compared to their counterparts, who exhibited a fall in methacholine (r=0.33; p>0.05). The four subjects in each correlation between PD35,s asthmatics (p<0.05). For the two groups, there was no methacholine in normal subjects. Indeed, PAF was on average 33 fold more potent than activating factor; PD 35: provocative dose of PAF or methacholine causing a 35% fall in specific airway conductance (Gaw). The comparison of PD35,s Gaw for PAF and methacholine in normal and asthmatic subjects.

### Table 4. – Comparison of PD35,s Gaw for PAF and methacholine in normal and asthmatic subjects

|        | PD35 PAF | PD35 Metha | PD35 PAF/Metha |
|--------|----------|------------|----------------|
| normals |          |            |                |
|        | 28       | 397        | 0.07           |
|        | 73       | 582        | 0.12           |
|        | 75       | 2142       | 0.02           |
|        | 34       | 8010       | 0.004          |
|        | 54       | 7500       | 0.007          |
|        | 200      | 229        | 0.87           |
|        | 132      | 4255       | 0.03           |
|        | 20       | 1530       | 0.01           |
| geom mean | 59       | 1622       | 0.03           |

| asthmatics |          |            |                |
|           | 39       | 269        | 0.18           |
|           | 12       | 61         | 0.20           |
|           | 25       | 56         | 0.45           |
|           | 27       | 158        | 0.17           |
|           | 34       | 127        | 0.27           |
|           | 87       | 137        | 0.63           |
|           | 21       | 689        | 0.03           |
|           | 20       | 179        | 0.11           |
| geom mean | 28*      | 148***     | 0.19*          |

*: p<0.05; ***: p<0.0001, asthmatics vs normals. PAF: platelet-activating factor; PD35: provocative dose of PAF or methacholine causing a 35% fall in specific airway conductance (Gaw); Geom mean: geometric mean.

Indeed, PAF was on average 33 fold more potent than methacholine in normal subjects versus 5.2 fold in asthmatics (p<0.05). For the two groups, there was no correlation between PD35,s Gaw PAF and PD35,s Gaw methacholine (r=0.33; p>0.05). The four subjects in each group who did not show a fall in Gaw of at least 35% during the full PAF challenge, did not have a significantly different geometric mean PD35,s Gaw methacholine compared to their counterparts, who exhibited a fall in sGaw of at least 35% on the full PAF challenge, (110 and 2,344 nmol in asthmatic and normal subjects, respectively). When calculating by interpolation the percentage fall in FEV1 for a 35% fall in sGaw in asthmatic versus normal subjects, a fall of 11.5±3.7% vs 2.6±0.6% (p<0.05) was found for methacholine and a fall of 7.2±2.5% vs 2.2±1% (p=0.09) for PAF.

**Discussion**

Our data clearly indicate the existence of a bronchial tachyphylaxis in vivo to PAF both in asthmatic and normal subjects. Second, although causing a greater airway obstruction in asthmatic than in normal subjects, PAF is less sensitive than methacholine at discriminating between the two groups.

Inhalation of a single dose of 60 µg PAF produced a more severe decrease in airway calibre, as assessed by sGaw, than that achieved when the same dose was inhaled during a full bronchial challenge. This demonstrates the tachyphylaxis of the airway tract to this mediator and confirms previous data obtained on isolated human airways [8]. This phenomenon was observed in both groups and was especially marked in the normal subjects. The shape of the one day PAF dose-response curve contrasts with that obtained for methacholine. This fact shows that the tachyphylaxis to PAF does not reflect an intrinsic and general property of the bronchial responsiveness of our subjects but a special responsiveness to this mediator.

The discrepancy between our results and those of Ruben et al. [6], who showed a dose-dependent fall in sGaw both in asthmatic and normal subjects, might be related to the difference in scale in the increments of doses inhaled. Indeed, doubling doses of PAF were used in the present study, whereas the subjects successively inhaled 10 fold increasing doses in the study by Rubin et al. [6]. Our data agree with those of Hoppe et al. [14], who reported a still more striking tachyphylaxis when performing PAF dose-response curve using a scale of doses very similar to ours. By contrast, Hseih [13] found a clear dose-dependent fall in FEV1 in asthmatic children inhaling successively doubling concentrations of PAF. This suggests that children might lack the tachyphylaxis usually observed in adults. The explanation for the tachyphylaxis seen after inhalation of PAF is not clear but could be due to a rapid internalization of the PAF receptors on the surface of the resident cells, possibly involved in the release of secondary bronchoconstricting mediators. Several studies performed both in vitro and in vivo suggest that leukotrienes may be involved [19–22]. An alternative explanation for the tachyphylaxis might be the fact that the first dose of PAF causes a plasmatic exudation in the bronchial mucosa [23], causing a rapid metabolism by acetyl-hydrolase [1] of any further dose.
potent than methacholine in asthmatics, which indicates a relative hyposensitivity to PAF in asthma. Such a relative hyposensitivity of asthmatics has been described previously, with leukotrienes as compared to histamine [24] or methacholine [25], which further supports the possibility that leukotrienes may mediate the PAF effect. In addition, given the tachyphylaxis which occurs after repeated inhalations of PAF, asthmatic subjects might acquire a slight desensitization as a result of a chronic in vivo exposure to this mediator. In this respect, PAF has been detected in BAL fluid of stable asthmatics but not normal subjects [2], and recent data using a new PAF-antagonist showed that endogenous PAF contributes to the basal bronchial hyperresponsiveness of asthmatics [26].

The lack of correlation between the PD35,s Gaw PAF and PD35,s Gaw methacholine confirms previous data obtained with different functional indices [11, 12] and emphasizes the difference in the mechanism of action of these mediators.

The PD35,s Gaw PAF of our asthmatic subjects was only twofold lower than that of normal subjects. Therefore, the difference in bronchial sensitivity in terms of change in s Gaw is weak. However, the maximal decrease in s Gaw after the PAF challenge was clearly greater in asthmatics, which indicates a higher reactivity. Thus, asthmatics may differ from normal subjects in other respects than an increased sensitivity of their proximal bronchi in response to a stimulus. Possibly, more characteristic of the asthmatic subjects is their incapacity to limit the extent of a bronchoconstriction once this has started, so that the s Gaw can reach a sufficiently low level resulting in a significant fall in FEV1. The abnormal physiological response of asthmatic bronchi is likely to be related to changes in the structural bronchial wall. The existence of an inflammatory process in the airway tract of asthmatics is well-established [27], and the presence of a presumably increased amount of liquid within the bronchial wall of asthmatic patients might exaggerate the decrease in airway lumen resulting from smooth muscle contraction for several reasons [28]. Furthermore, we found that, even for the same 35% decrease in s Gaw, the corresponding fall in FEV1 was higher in asthmatic than in normal subjects irrespective of the mediator used for the bronchial challenge. This indicates that asthmatics have a special trend to impair their expiratory flow rates during a forced expiration. A lack of bronchodilation during a deep inspiration prior to forced expiration might be of paramount importance [29]. Furthermore, a reduced elastance of the extracellular matrix within the bronchial wall of asthmatics [30] might play an additional role, by facilitating bronchial collapse during a forced dynamic compression. Thus, even if the bronchial sensitivity to PAF assessed in terms of the threshold dose causing a 35% fall in s Gaw is not strikingly different between asthmatic and normal subjects, the consequences of a PAF bronchial challenge in terms of the impairment in the expiratory flow rates are clearly more marked in the asthmatic subjects.

In summary, although asthmatics develop greater bronchial obstruction after inhalation of platelet-activating factor than normal subjects, methacholine is much more sensitive than platelet-activating factor at discriminating between asthmatic and normal subjects. Since our data show a bronchial tachyphylaxis to platelet-activating factor, the relative hyposensitivity in asthma might be the result of a chronic in vivo exposure of bronchi to endogenously produced platelet-activating factor. Further studies are needed to clarify the way in which platelet-activating factor induces bronchospasm in man, and especially in asthmatics.

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