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Acute bronchial obstruction following inhalation of PAF in asthmatic and normal subjects: comparison with methacholine

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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 (sG_{aw}) and forced expiratory volume in one second (FEV_1) 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 sG_{aw} and FEV_1 to plateau after the second dose in each group, whereas methacholine provoked a dose-related decrease in sG_{aw} and FEV_1. 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 sG_{aw} 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 sG_{aw} and FEV_1 after PAF inhalation were significantly higher in the asthmatics than in the normal subjects. The provocative dose of PAF causing a 35% fall in sG_{aw} ($PD_{35,sG_{aw}}$) 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 $PD_{35,sG_{aw}}$ 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 ($p<0.05$). The percentage falls in FEV_1 (calculated by interpolation) for a 35% fall in sG_{aw}, were greater in asthmatics than in normals both for methacholine ($p<0.05$) 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.

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Platelet-activating factor (PAF) is a potent inflammatory mediator produced 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 (sG_{aw}), expiratory flow rate at 30% of vital capacity on a partial flow-volume curve (V'_{p30}), and forced expiratory volume in one second (FEV_1). 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 FEV_1 after the last dose. CHUNG and BARNES [11] found a comparable fall in V'_{p30} in mild asthmatics and normal subjects after inhalation of doses ranging 12–24 µg. By contrast, using both sG_{aw} and forced expiratory flow rates to assess the bronchial response, we have recently demonstrated a clearly more marked acute airway obstruction

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 FEV_1 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 FEV_1 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 FEV_1 (PC_{20},FEV_1)) 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 FEV_1 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.

Table 1. – Patient characteristics

Subject No.	Age yrs	Sex	Tobacco	Atopy	FEV_1 % pred	PC_{20} 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+ICoR
9	21	F	No	Yes	86	0.07	SO+ICoR
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; FEV_1: forced expiratory volume in one second; PC_{20}: provocative concentration of methacholine causing a 20% fall in FEV_1; 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 PC_{20} methacholine was determined according to the method described by COCKROFT *et al*. [18], starting with 0.03 and 1 $mg \cdot mL^{-1}$ in asthmatic and normal subjects, respectively, and reaching maximally 16 $mg \cdot mL^{-1}$. 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 $mg \cdot mL^{-1}$ (18 µg aerosolized), up to at least 0.25 $mg \cdot mL^{-1}$ (144 µg aerosolized). Measurements of $_{s}G_{aw}$ (Plethysmography; Bodytest, Jaeger) and FEV_1 (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 $_{s}G_{aw}$ was recorded as the mean of three values, whilst FEV_1 was recorded as the best of three manoeuvres. If a fall of at least 35% $_{s}G_{aw}$ had not occurred after inhalation of 0.25 $mg \cdot mL^{-1}$, the test was carried on by doubling the dose until this threshold was reached. The $PD_{35,s}G_{aw}$ was interpolated from the dose-response curve. Any fall in FEV_1 higher than 20% was reversed at the end of the test by 400 µg inhaled salbutamol. If the FEV_1 had dropped by more than 30%, the subject was kept under medical control for 1 h after the challenge and released afterwards when the FEV_1 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 $\mu g \cdot mL^{-1}$) were prepared as described previously [12] from a vial containing 2 $mg \cdot mL^{-1}$ solution in chloroform. Each stock solution contained 2% ethanol, was kept at 4°C and used within 48 h. Both the nebulizer and the procedure of inhalation (2 min, tidal breathing) used for the PAF challenge were similar to those used for methacholine challenge.

On Visit 2 of the protocol, the subjects inhaled doubling doses of PAF ranging 15–120 µg every 20 min. Measurements of $_{s}G_{aw}$ and FEV_1 were performed 5, 10 and 15 min after each dose, using the same procedure as described above with methacholine, and the lowest value was retained for drawing the dose-response curve. The variations in functional index were expressed as a percentage of control value, which was the value observed after inhalation of a solution of isotonic saline with 2% ethanol. If the fall in $_{s}G_{aw}$ had reached at least 35% from control, the $PD_{35,s}G_{aw}$ was calculated by interpolation.

On Visit 3 and 4 of the protocol, the subjects inhaled 30 and 60 µg PAF, respectively. The measurements of $_{s}G_{aw}$ and FEV_1 were performed 5, 10 and 15 min after PAF inhalation and the variations expressed as a percentage of control value. The lowest values observed at these different time-points were retained to draw the dose-response curve of PAF challenges performed on different days (from 15 to 60 µg). Since the bronchial response occurring after the first dose of PAF inhaled on Visit 1 could be considered as a response to a single dose, the values obtained after 15 µg on Visit 1 were used again to construct the "PAF different days dose-response curve". Any fall in FEV_1 >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 $_{s}G_{aw}$ and FEV_1 on the different study days were assessed by two-way analysis of variance (ANOVA). The significance of the decrease in $_{s}G_{aw}$ and FEV_1 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 $_{s}G_{aw}$ and FEV_1 after the different doses between asthmatic and normal subjects was made by using an unpaired t-test. The comparison of the $PD_{35,s}G_{aw}$ PAF or $PD_{35,s}G_{aw}$ 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 $_{s}G_{aw}$ and FEV_1 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 $PD_{35,s}G_{aw}$ PAF and $PD_{35,s}G_{aw}$ 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 FEV_1 or $_{s}G_{aw}$ between the asthmatic and normal subjects on the different study days (two-way ANOVA) (table 2).

Successive 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 FEV_1 and $_{s}G_{aw}$, 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 $_{s}G_{aw}$ did not increase significantly further after 15 µg, and the variations in FEV_1 were clinically irrelevant (≤5%) at all doses of PAF tested. Significant falls from baseline both in FEV_1 (8.3±2.9%; $p<0.05$) and $_{s}G_{aw}$ (30±5.4%; $p<0.001$) were already achieved with inhalation of 15 µg PAF in the asthmatic group, while the fall in $_{s}G_{aw}$ became statistically significant after 30 µg inhaled PAF in the normal subjects (25±6.4%; $p<0.01$). The falls in FEV_1 were significantly higher in the asthmatic than in the normal subjects at all doses of PAF tested, while significant differences for the falls in $_{s}G_{aw}$ 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

Table 2. – Baseline lung function on the different study days

	Visit 1 methacholine	Visit 2 full PAF	Visit 3 PAF 30 µg	Visit 4 PAF 60 µg
Asthmatics				
sG_{aw} $kPa^{-1}\cdot s^{-1}$	2.10 (0.17)	2.14 (0.40)	1.91 (0.23)	2.18 (0.26)
FEV_1 L	3.51 (0.27)	3.68 (0.36)	3.50 (0.35)	3.46 (0.31)
Normals				
sG_{aw} $kPa^{-1}\cdot s^{-1}$	2.79 (0.30)	2.78 (0.52)	2.52 (0.31)	2.76 (0.28)
FEV_1 L	3.78 (0.17)	3.90 (0.18)	3.83 (0.20)	3.85 (0.18)

Values are presented as mean, and SEM in parenthesis. sG_{aw}: specific airway conductance; PAF: platelet-activating factor; FEV_1: forced expiratory volume in one second.

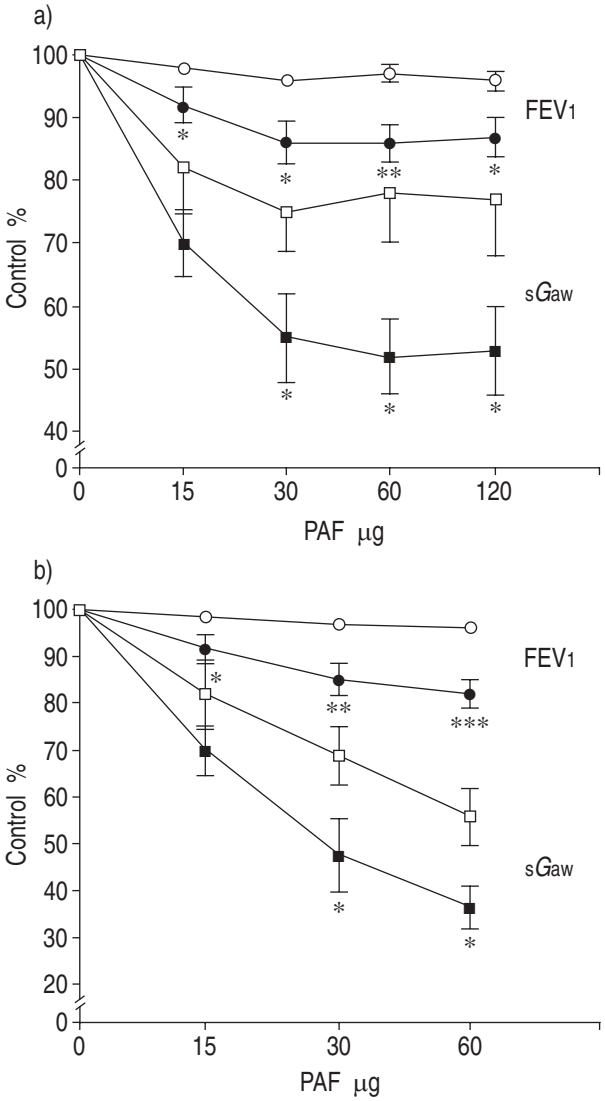

Fig. 1. – a) Dose-response curves of inhaled PAF-induced airway constriction in asthmatic (closed symbols) and normal subjects (open symbols) for each dose of PAF given during the same challenge. b) Dose-response curves of PAF-induced airway obstruction in asthmatic and normal subjects for each dose of PAF given on separate days. Squares: specific airway conductance; circles: forced expiratory volume in one second. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$, for asthmatic *vs* normal subjects. Error bars are omitted where graphically too small. PAF: platelet-activating factor.

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 sG_{aw} 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 FEV_1 after 60 µg in asthmatics tended to be greater than that after 15 µg ($p=0.06$). The falls in FEV_1 were significantly more pronounced in asthmatics than in normal subjects for all doses tested. The falls in sG_{aw} were significantly higher in asthmatics after inhalation of 30 and 60 µg.

Both in asthmatic and normal subjects, the maximal decreases in sG_{aw} after 60 µg PAF inhaled as a single dose 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 FEV_1 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 FEV_1 and sG_{aw}.

$PD_{35,sG_{aw}}$ PAF could be determined in eight subjects of each group during the full bronchial challenge (table 4). The $PD_{35,sG_{aw}}$ PAF was slightly but significantly lower in the asthmatics ($p<0.05$). In the same subjects, $PD_{35,sG_{aw}}$ 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 FEV_1	17±3	14±3	>0.05
Fall in sG_{aw}	64±5	48±6	<0.05
Normals			
Fall in FEV_1	3.6±0.8	3.2±1.4	>0.05
Fall in sG_{aw}	44±6	22±8	<0.001

#: 105 µg cumulative dose. Values are presented as mean±SEM. For abbreviations see legend to table 2.

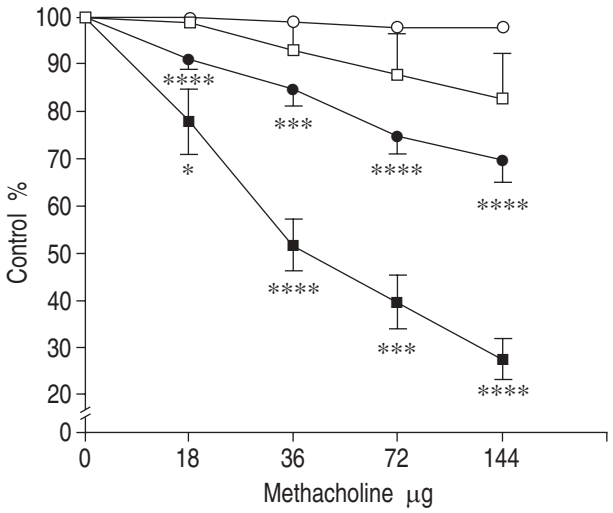

Fig. 2. – Dose-response curves of methacholine-induced airway obstruction in asthmatic (closed symbols) and normal subjects (open symbols). Squares: specific airway conductance; circles: forced expiratory volume in one second. ***: p<0.001; ****: p<0.0001, for asthmatic *vs* normal subjects.

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 $PD_{35,sGaw}$ PAF and $PD_{35,sGaw}$ methacholine (r=0.33; p>0.05). The four subjects in each group who did not show a fall in $sGaw$ of at least 35% during the full PAF challenge, did not have a significantly different geometric mean $PD_{35,sGaw}$ 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 FEV_1 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.

Table 4. – Comparison of $PD_{35,sGaw}$ for PAF and methacholine in normal and asthmatic subjects

	PD_{35} PAF nM	PD_{35} Metha nM	PD_{35} 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	209	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<0.001, asthmatics *vs* normals. PAF: platelet-activating factor; PD_{35}: provocative dose of PAF or methacholine causing a 35% fall in specific airway conductance ($sGaw$); Geom mean: geometric mean.

Discussion

Our data clearly indicate the existence of a bronchial tachyphylaxis *in vivo* to PAF both in asthmatic and normal subjects. Secondly, 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 RUBIN *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 HOPP *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, HSIEH [13] found a clear dose-dependent fall in FEV_1 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 metabolization by acetyl-hydrolase [1] of any further dose of PAF locally delivered.

The comparison of $PD_{35,sGaw}$ for PAF and methacholine in 8 of the 12 subjects in each group demonstrates that methacholine, which is a direct constrictor of airway smooth muscle, is much more powerful than PAF in discriminating between the bronchial responsiveness of asthmatic and normal subjects. Whilst being on a molar basis 33 times more potent than methacholine in normal subjects, PAF is only five times more

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 $PD_{35,sGaw}$ PAF and $PD_{35,sGaw}$ methacholine confirms previous data obtained with different functional indices [11, 12] and emphazises the difference in the mechanism of action of these mediators.

The $PD_{35,sGaw}$ 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 $sGaw$ is weak. However, the maximal decrease in $sGaw$ 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 $sGaw$ can reach a sufficiently low level resulting in a significant fall in FEV_1. 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 $sGaw$, the corresponding fall in FEV_1 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 $sGaw$ 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 endogeneously 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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