

Differences in airway responsiveness to acetaldehyde and methacholine in asthma and chronic bronchitis

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ABSTRACT: Inhaled acetaldehyde may induce bronchoconstriction in asthmatic subjects and provides a new method to investigate airway responsiveness. The objective of the study was to determine whether acetaldehyde was a more specific stimulus than methacholine in differentiating asthma from chronic bronchitis with or without airflow limitation.

Bronchial provocation challenges with methacholine and acetaldehyde were performed in 62 asthmatics and in 59 smokers with chronic bronchitis (32 with chronic bronchitis alone and 27 with chronic bronchitis and coexisting chronic obstructive pulmonary disease (COPD)). The response to both bronchoconstrictor agents was measured by the provocative concentration required to produce a 20% fall in forced expiratory volume in one second (FEV₁; PC₂₀).

The two types of challenge yielded a similarly high level of sensitivity (100% for methacholine and 92% for acetaldehyde) in revealing airway hyperresponsiveness in asthma. However, bronchoprovocation with acetaldehyde yielded considerably greater specificity (95%) than bronchoprovocation with methacholine (24%) in separating asthma from chronic bronchitis. In subjects with asthma, methacholine and acetaldehyde responsiveness were weakly but significantly correlated ($r=0.42$, $p=0.001$) but no correlation was found between airway responsiveness to acetaldehyde and baseline FEV₁ ($r=0.13$, $p=0.33$).

These findings suggest that the demonstration of bronchoconstriction in response to acetaldehyde may be a more specific test than methacholine in the differentiation of asthma from chronic bronchitis. Furthermore, methacholine and acetaldehyde hyperresponsiveness are not reflecting the same pathophysiological process in the airways. *Eur Respir J 2000; 15: 260–265.*

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Airway hyperresponsiveness (AHR) is usually regarded as an abnormal response of the lower respiratory tract to a number of nonsensitizing bronchoconstrictive stimuli [1]. These stimuli can be divided into those which are thought to act mainly through a direct effect on airway smooth muscle (e.g. methacholine and histamine), and those which may exert an indirect effect on airway smooth muscle cells (e.g. isocapnic hyperventilation, hyperosmolar saline, adenosine). Methacholine or histamine challenge has been widely used for the detection and quantitation of AHR in asthmatic patients [2, 3]. Some authors suggested a cut-off limit of 8 mg·mL⁻¹ for the provocative concentration causing a 20% fall in forced expiratory volume in one second (FEV₁; PC₂₀) methacholine [3] or PC₂₀ histamine [2], since all their nonasthmatic subjects had a PC₂₀ above this value while all their asthmatic subjects with recent symptoms had a lower PC₂₀ value. However, it is evident that many smokers with chronic bronchitis or chronic obstructive pulmonary disease (COPD) also had AHR to inhaled histamine or methacholine [4–9]. Therefore, measurements of airway responsiveness to direct bronchoconstrictors do not appear to be useful to distinguish asthma from COPD or other respiratory disorders [10, 11].

Airway responsiveness also can be measured by stimuli that appear to cause bronchoconstriction through the release of mediators from cells within the airways, and these may be preferable for distinguishing between patients with asthma and those with COPD [12, 13]. The inhalation of acetaldehyde may induce bronchoconstriction in asthmatic subjects and it provides a new method to investigate airway responsiveness [14]. Although the mechanism by which inhalation of acetaldehyde leads to bronchoconstriction remains to be determined, a role for mast cells (or basophils) mediator release has been suggested [15, 16]. Data on airway responsiveness to inhaled acetaldehyde come from studies with small sample sizes. MYOU *et al.* [14] reported that the airways of nine asthmatic patients were more responsive to acetaldehyde than those of nine healthy control subjects. However, no previous information is available on differences in airway responsiveness to inhaled acetaldehyde between asthmatic subjects and patients with chronic bronchitis with or without COPD.

In asthma, airway responsiveness has been associated with airway inflammation [17, 18]. Like asthma, chronic bronchitis in smokers is also associated with an infiltration of the airway wall by inflammatory cells [19–24].

In a proportion of bronchitics this may result in chronic damage to the airway wall and destruction of lung parenchyma with consequent reduction of airway calibre, increased resistance to airflow, and loss of lung elastic recoil referred to as COPD [25]. However, the critical features which determine AHR in subjects with chronic bronchitis are not yet determined. If inflammatory processes are involved in the pathogenetic mechanisms leading to AHR in asthma and chronic bronchitis, bronchial challenge testing using indirect stimuli may be preferable to challenges with direct stimuli for the differential diagnosis between asthma and chronic bronchitis.

The object of the study was to determine whether acetaldehyde was a more specific stimulus than methacholine in differentiating asthma from chronic bronchitis with or without airflow limitation.

Subjects and methods

Subject

Sixty-two nonsmoking patients with mild asthma and 59 current smokers with chronic bronchitis were recruited from the outpatient clinic of the Department of Pulmonary Diseases, Hospital Arnau de Vilanova, Valencia, Spain. The study protocol was approved by the Ethics Committee of the hospital. All participants were informed of the aim of the study, and they gave written consent.

Diagnosis of asthma and chronic bronchitis was performed according to the American Thoracic Society criteria [26, 27]. Asthmatic subjects were defined as those individuals with a characteristic asthmatic history (recurrent attacks of reversible dyspnoea with wheezing) and who had also previously had at least one of the following criteria: an increase in FEV₁ of at least 15% after inhalation of 200 µg of salbutamol, or a positive methacholine challenge test, defined as a PC₂₀ ≤ 8 mg·mL⁻¹ [2]. Further selection criteria at enrolment in the study were: 1) age between 18–60 yrs; 2) FEV₁ ≥ 80% predicted. Chronic bronchitis was defined as the presence of chronic productive cough for at least 3 months a year during at least the previous 2 yrs. All subjects had chest radiographs; those who showed changes of emphysema were excluded. Further selection criteria at enrolment in the study were as follows: 1) age 40–60 yrs; 2) a history of cigarette smoking; 3) FEV₁ ≥ 60% predicted; 4) no past history of asthma or allergic rhinitis; 5) an increase in FEV₁ < 12% from baseline after inhalation of 200 µg salbutamol.

All subjects of both groups had been free of acute respiratory tract infections within the preceding month. Subjects with significant renal, hepatic or cardiovascular disease, and pregnant females were excluded. None of the subjects had received oral corticosteroids within 2 months before the study. Inhaled corticosteroids, sodium cromoglycate, and nedocromil sodium, if used, were stopped at least 4 weeks before the onset of the study. Theophylline and β₂-agonists were withdrawn as follows: long-acting inhaled β₂-agonists and theophylline for 24 h and short-acting inhaled β₂-agonists for 6 h.

Study design

The subjects attended the laboratory on four visits at the same time of day. On their first visit, all subjects were

evaluated for suitability and written consent was obtained after full explanation. On the second visit (1–30 days after initial evaluation), FEV₁ was measured before and 15 min after inhaling 200 µg of salbutamol through a metered dose inhaler. On day 3, a methacholine inhalation test was performed and PC₂₀ methacholine was determined, followed after 3–7 days (visit 4) by an acetaldehyde inhalation test. In all subjects the methacholine challenge test was performed first.

Methods

Pulmonary function. Spirometry was performed with a calibrated dry rolling seal spirometer (TT Auto Link; PK Morgan Instruments Inc., Andover, MA, USA) according to standardized guidelines [28]. Baseline FEV₁ and forced vital capacity (FVC) were measured until three reproducible recordings, differing < 5% were obtained. Highest values were used for analyses. The subject then inhaled 200 µg salbutamol *via* a Volumatic spacer (GlaxoWellcome, Madrid, Spain). After 15 min measurements of FEV₁ were repeated. Reference values were those of the European Community for Coal and Steel [29].

Methacholine and acetaldehyde inhalation tests. Inhalation provocation tests were performed according to a 2-min tidal breathing method adapted from COCKCROFT *et al.* [2] for methacholine and from MYOU *et al.* [14] for acetaldehyde. Solutions of methacholine (Sigma Chemical Co, St. Louis, MO, USA) and acetaldehyde (E. Merck, Darmstadt, Germany) were prepared in a 0.9% sodium chloride solution and were administered at room temperature as aerosols generated from a starting volume of 2 mL in a Hudson 1720 nebulizer (Hudson, Temecula, CA, USA). Nebulizer output was 0.18 ± 0.02 mL·min⁻¹. After inhalation of 0.9% sodium chloride solution, doubling concentrations of 0.095–25 mg·mL⁻¹ methacholine or 5–40 mg·mL⁻¹ acetaldehyde were inhaled. Due to the effect of a deep inspiration on subsequent airway tone [30], only one measurement for FEV₁ was performed 60–90 s after inhalation of each concentration unless the forced expiratory manoeuvre was judged to be technically unsatisfactory. The test was interrupted when FEV₁ dropped by > 20% or when the highest concentration had been administered.

Data analysis

Methacholine and acetaldehyde PC₂₀ values were calculated by linear interpolation between the last two data points of the logarithmic concentration-response curve. On the few occasions (four asthmatic subjects) during the acetaldehyde or methacholine challenge when the initial concentration produced a > 20% decrease in FEV₁ the provocative concentrations were back-extrapolated. Back-extrapolation was performed by constructing a straight line from the diluent point to the value obtained with the first administered concentration; PC₂₀ was defined as the point where that line intersected a horizontal line representing a 20% decrease in FEV₁. Participants were categorized as having AHR if they exhibited a PC₂₀ methacholine ≤ 8 mg·mL⁻¹ [2] or a PC₂₀ acetaldehyde ≤ 40 mg·mL⁻¹ [14]. Sensitivity (ability of each bronchoconstrictor to detect subjects with asthma in the population studied) and

specificity (ability of each bronchoconstrictor to detect subjects without asthma in the population studied) were evaluated.

A PC₂₀ value for methacholine could not be obtained in five subjects with chronic bronchitis because patients did not bronchoconstrict with the highest concentration of constrictor agent. Also, a PC₂₀ value for acetaldehyde could not be calculated in five subjects with asthma. On these occasions the PC₂₀ value was censored to the highest concentration of methacholine or acetaldehyde given (25 mg·mL⁻¹ and 40 mg·mL⁻¹, respectively). A PC₂₀ value for acetaldehyde could not be obtained in 56 patients with chronic bronchitis. All PC₂₀ values were log transformed for analysis and geometric mean values are given.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS for windows, release 6.01; SPSS Inc., Chicago, IL, USA). To evaluate normality of distributions Kolmogorov-Smirnov test was used and a p-value >0.05 was obtained. Thus, parametric analyses (Students' t-test to compare group means and Pearson r for correlations) were used. Categorical variables were analysed with the Fisher's exact test. All tests were performed two-sided and values of p<0.05 were considered significant.

Results

Patient characteristics

Table 1 shows the characteristics of the patients. Asthmatic subjects were younger than patients with chronic bronchitis (p<0.001), and the percentage of females was significantly greater in the group with asthma than in the group with chronic bronchitis (p<0.001). As expected (selection criteria), baseline FEV₁ per cent predicted and FEV₁/FVC percentage were also significantly greater in the asthmatic group than in the chronic bronchitis group (p<0.001). Mean baseline FEV₁ values were not significantly different within the two groups before the two different provocation tests (table 1).

Airway responsiveness to methacholine and acetaldehyde in asthmatics and chronic bronchitics

Individual results of the two challenges in the two groups are shown in figure 1. All subjects with asthma showed AHR to methacholine but five subjects (8%) failed to respond to acetaldehyde. In the chronic bronchitis group 45 subjects (76%) showed AHR to methacholine, whereas only three patients (5%) showed AHR to acetaldehyde.

Table 1. – Subject characteristics

	Asthma	Chronic bronchitis
n	62	59
Age yrs	31±1	56±1
Per cent male	39	88
Pack-years	-	51.1±3.4
FEV ₁ % predicted	97.4±1.4	83.2±2.0
FEV ₁ /FVC %	78.2±1.0	69.0±1.2
Prechallenge FEV ₁ L		
Methacholine	3.39±0.11	2.45±0.07
Acetaldehyde	3.33±0.10	2.42±0.07

Data are presented as absolute numbers and mean±SEM. FEV₁: forced expiratory volume in one second; FVC: forced vital capacity.

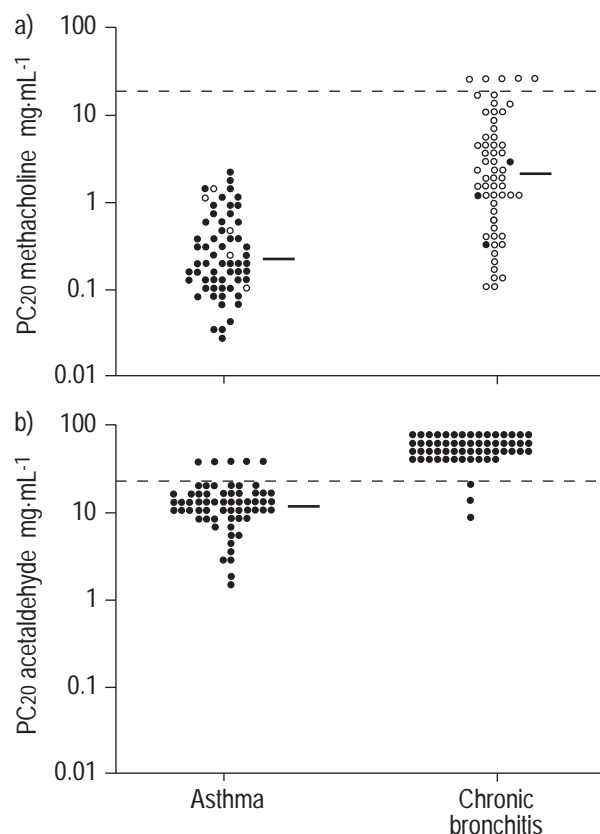


Fig. 1. – a) Provocative concentration causing a 20% fall in forced expiratory volume in one second (FEV₁; PC₂₀) methacholine values in subjects with (●) and without (○) airway hyperresponsiveness to acetaldehyde. PC₂₀ acetaldehyde values of subjects with asthma and patients with chronic bronchitis. Horizontal solid lines indicate the geometric means. Dashed lines indicate the highest concentration administered.

Although sensitivity for acetaldehyde (92%) was a little less than for methacholine (100%) in revealing AHR in asthma, bronchoprovocation with acetaldehyde yielded considerably greater specificity (95%) than bronchoprovocation with methacholine (24%) in separating asthma from chronic bronchitis. Therefore, bronchoprovocation with acetaldehyde was a more accurate diagnostic test (93%) than bronchoprovocation with methacholine (60%) to differentiate asthma and chronic bronchitis.

The geometric mean PC₂₀ methacholine in the asthma group (0.29 mg·mL⁻¹) was significantly lower than in the chronic bronchitis group (2.09, p<0.001). Despite the observed differences in PC₂₀ values, there was considerable overlap in individual PC₂₀ levels between the two groups (fig. 1).

In the chronic bronchitis group 56 of the 59 subjects did not respond to the highest acetaldehyde concentration administered and this precluded comparison of the PC₂₀ acetaldehyde between asthmatics and patients with chronic bronchitis.

PC₂₀ methacholine and PC₂₀ acetaldehyde were significantly correlated in the asthma group (r=0.42, p=0.001). Side effects after inhalation of acetaldehyde were cough, chest tightness, and pharyngeal irritation. No other side effects were documented.

Relations between airway responsiveness and baseline forced expiratory volume in one second

PC₂₀ methacholine and FEV₁ % predicted were significantly correlated in the chronic bronchitis group ($r=0.28$, $p=0.03$), but no correlation was found between these two functions in the asthmatic subjects ($r=0.18$, $p=0.16$).

No correlation was found between airway responsiveness to acetaldehyde and FEV₁ % predicted in asthmatics ($r=0.13$, $p=0.33$; fig. 2). However, interpretation of the results is limited by the small range of FEV₁ % predicted (80–122%)

Differences in airway responsiveness between subjects with chronic bronchitis alone and patients with chronic obstructive pulmonary disease

Of the 59 smokers with chronic bronchitis 32 had chronic bronchitis alone (FEV₁/FVC% $\geq 70\%$ and FEV₁ $\geq 80\%$ predicted), whereas 27 had COPD (FEV₁/FVC% $< 70\%$ or FEV₁ $\leq 79\%$ predicted). Subjects with COPD had significantly lower baseline values for FEV₁ % predicted ($74.3 \pm 1.8\%$) than those with chronic bronchitis alone ($90.4 \pm 2.8\%$, $p < 0.001$).

Individual results for the two challenges in the two groups are shown in figure 3. The proportion of subjects with AHR to methacholine was lower in subjects with chronic bronchitis alone (62%) than in patients with COPD (89%, $p=0.03$). There were no differences in the proportion of subjects with AHR to acetaldehyde among the two groups (7% in patients with COPD and 3% in subjects with chronic bronchitis alone, $p=0.59$).

The geometric mean PC₂₀ methacholine value in the COPD group ($1.35 \text{ mg}\cdot\text{mL}^{-1}$) was significantly lower than in the group with chronic bronchitis alone ($3.09 \text{ mg}\cdot\text{mL}^{-1}$, $p=0.04$).

Discussion

In this study, the authors have found that bronchoprovocation with acetaldehyde is of more value than bronchoprovocation with methacholine in the differentiation of asthma from chronic bronchitis with or without airflow

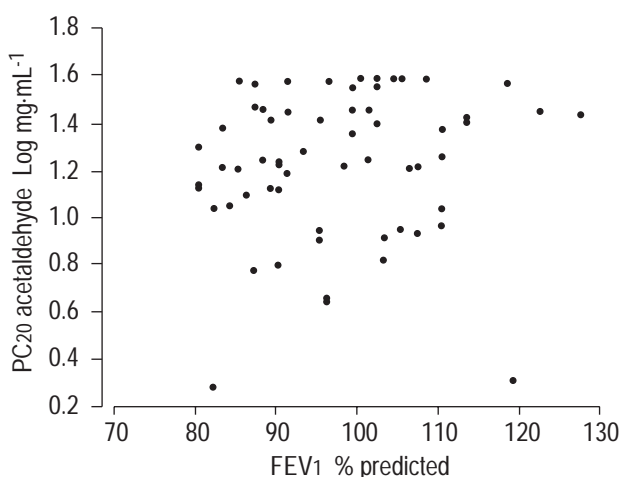


Fig. 2. – Correlation between airway responsiveness to acetaldehyde and baseline forced expiratory volume in one second (FEV₁) in subjects with asthma ($r=0.13$, $p=0.33$). Individual values are shown. PC₂₀: provocative concentration causing 20% fall in FEV₁.

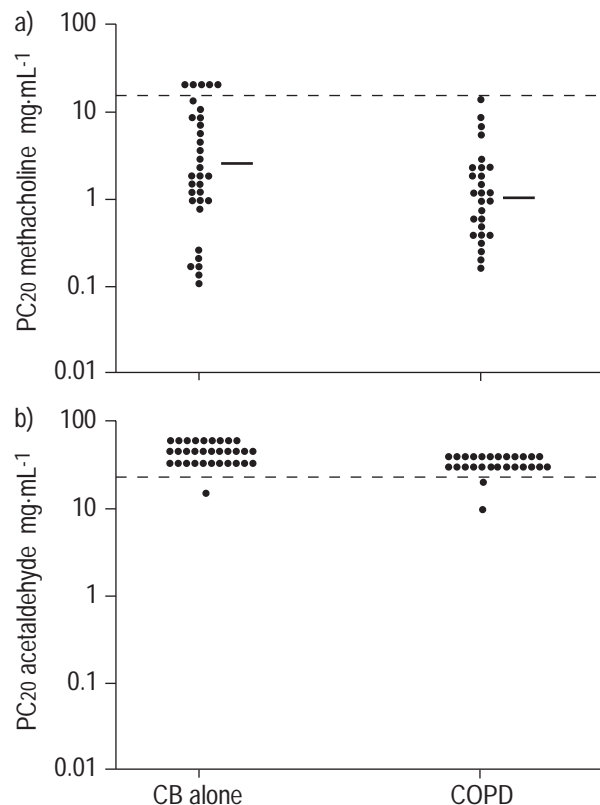


Fig. 3. – Provocative concentration causing a 20% fall in forced expiratory volume in one second (FEV₁; PC₂₀) methacholine (a) and PC₂₀ acetaldehyde (b) values of subjects with chronic bronchitis (CB) alone and patients with chronic obstructive pulmonary disease (COPD). Horizontal solid lines indicate the geometric means. Dashed lines indicate the highest concentration administered.

limitation. Furthermore, this study shows that asthmatic patients develop bronchoconstriction in response to acetaldehyde and that there is a significant correlation between responsiveness to acetaldehyde and methacholine. This is in contrast to patients with chronic bronchitis who in general did not develop bronchoconstriction with acetaldehyde despite an increased responsiveness to methacholine. These findings suggest that the pathogenetic mechanism leading to AHR is different in patients with asthma and with chronic bronchitis.

To the best of the authors' knowledge, no previous information is available on differences in airway responsiveness to inhaled acetaldehyde between asthmatics and patients with chronic bronchitis. In the present study, a fall in FEV₁ of $> 20\%$ was detected in 92% of the subjects with asthma after inhalation of acetaldehyde in concentrations up to $40 \text{ mg}\cdot\text{mL}^{-1}$, whereas this was detected in only three patients (5%) with chronic bronchitis. In contrast, a strikingly high proportion (76%) of the subjects with chronic bronchitis exhibited AHR to methacholine, in agreement with previous observations by others of AHR to pharmacological agents (histamine or methacholine) in chronic bronchitis with or without airflow limitation [4–9]. However, taken as a group, the degree of methacholine responsiveness was significantly less, (higher PC₂₀ values) than that found in the group of asthmatics. The present report, by showing that inhaled acetaldehyde causes bronchoconstriction in subjects with asthma, is in keeping with

the results of MYOU *et al.* [14] who reported that acetaldehyde provoked concentration related bronchoconstriction when administered by inhalation to asthmatic subjects. In addition, the present study is the first showing that inhaled acetaldehyde causes bronchoconstriction in a very small proportion of patients with chronic bronchitis.

The mechanism underlying the bronchoconstriction caused by inhalation of acetaldehyde in patients with asthma is not certain. In a series of studies conducted in asthmatic subjects, premedication with H₁-histamine receptor antagonists [14, 31] and selective inhibitors of thromboxane synthetase [16] have been shown to substantially inhibit the acute bronchoconstrictor response to inhaled acetaldehyde. Therefore, it is unlikely that acetaldehyde acts directly on smooth muscle cells *in vivo*, but indirectly through activation of inflammatory cells in the airways [14–16, 31]. Exactly which cells are stimulated by acetaldehyde to initiate processes leading to smooth muscle contraction is as yet uncertain. Although this study was not designed to examine the mechanism of action of acetaldehyde, the weak correlation that was observed between methacholine responsiveness and acetaldehyde responsiveness, lends indirect support to the suggestion that acetaldehyde and methacholine exert their bronchoconstrictor effects through mechanisms that are at least partially different.

Over the last few years, evidence has accumulated to support the theory that airway inflammation is responsible for structural changes that determine AHR in asthma [32]. Although the critical features that determine AHR in subjects with chronic bronchitis are not yet determined, evidence is now growing that airway inflammation may also play an important role in the pathogenesis of chronic bronchitis [19, 20] or COPD [21–23]. However, in contrast to asthma the predominant type of inflammatory cell and the main anatomical site of the lesion in chronic bronchitis and COPD appear to differ [24]. Therefore, if release of bronchoconstrictive mediators by mast cells, basophils or other inflammatory cells [14, 16, 31] is the main pathway by which acetaldehyde is acting, the results of this study suggest that the bronchoconstrictor response to acetaldehyde involves one pathway which may be abnormally activated in asthma, but not in chronic bronchitis.

The primary aim of the study was to determine whether acetaldehyde was a more specific stimulus than methacholine in differentiating asthma from chronic bronchitis. From the present results, it is evident that sensitivity for acetaldehyde (92%) was a little less than that for methacholine (100%) in revealing AHR in asthma. In contrast, bronchoprovocation with acetaldehyde yielded considerably greater specificity (95%) than bronchoprovocation with methacholine (24%) in separating asthma from chronic bronchitis. From these comparisons it appears that acetaldehyde is of much more value than methacholine in the differentiation of asthma from chronic bronchitis or COPD. However, although the demonstration of bronchoconstriction in response to acetaldehyde may be a more specific test than methacholine in the differentiation of asthma from chronic bronchitis, there is still the possibility that asthma could be missed in a few individuals.

All of the subjects with >20% fall in FEV₁, after inhalation of acetaldehyde showed AHR to methacholine, but 47 subjects with a positive methacholine challenge

failed to respond to acetaldehyde. Therefore, in those patients with AHR to acetaldehyde the authors have always observed hyperresponsiveness to methacholine. By contrast, AHR to methacholine is not necessarily accompanied by hyperresponsiveness to acetaldehyde. In addition, the lack of response to acetaldehyde in smokers with chronic bronchitis is in marked contrast to findings in asthmatic subjects. In this study, 57/62 asthmatic subjects with a PC₂₀ methacholine between 0.04–4.33 mg·mL⁻¹ experienced a 20% fall in FEV₁ in response to acetaldehyde. On the contrary, only three of the 39 smokers with chronic bronchitis who had a PC₂₀ methacholine in a similar range (0.10–4.14 mg·mL⁻¹) showed AHR to acetaldehyde (fig. 1). These results suggest that methacholine hyperresponsiveness is a necessary, but not sufficient precondition for the expression of AHR to acetaldehyde, and that methacholine and acetaldehyde hyperresponsiveness are not reflecting the same pathophysiological process in the airways.

The authors acknowledge that a potential weakness of this study is that inhalation challenges were not performed randomly. It has been reported that inhaled acetaldehyde increases airway responsiveness to methacholine in asthmatic subjects [33], and for that reason the methacholine challenge test was performed first.

Within the group of subjects with chronic bronchitis, there was a significant correlation between PC₂₀ methacholine and baseline FEV₁. This is in keeping with previous reports [4, 5, 7, 8, 34]. By contrast, no correlation was found between methacholine or acetaldehyde responsiveness and baseline FEV₁ in asthmatics. However, the asthmatic subjects displayed a relatively narrow range of FEV₁ values. Thus, the authors cannot deduce with any certainty a conclusion from the correlation between baseline FEV₁, and methacholine or acetaldehyde responsiveness.

On the other hand, the proportion of subjects with AHR to methacholine was lower among subjects with chronic bronchitis alone than in those with COPD. This can probably be explained as a consequence of different baseline FEV₁ values between both groups, the values being lower in the COPD group. By contrast there were no differences in the proportion of subjects with AHR to acetaldehyde among the two groups. This may imply that in subjects with chronic bronchitis, methacholine (but not acetaldehyde) responsiveness is partially dependent on initial airway calibre.

In conclusion, this study demonstrates that bronchoprovocation with acetaldehyde is of much more value than bronchoprovocation with methacholine in the differentiation of asthma from chronic bronchitis. However, the tests have not been compared in a population of patients in whom the diagnosis was not previously established. This is necessary before the place of acetaldehyde in distinguishing between asthma and chronic bronchitis is truly identified.

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