Effect of challenge method on sensitivity, reactivity, and maximal response to methacholine

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Background: Recent data suggest that the tidal breathing method may produce methacholine provocation concentration that caused a decrease in forced expiratory volume in 1 second of 20% (PC_{20}) values significantly lower than the dosimeter method; however, the effect of the challenge method on the shape of the concentration-response curve has not been investigated.

Objective: To determine the effect of the challenge method on sensitivity, reactivity, and maximal response to methacholine.

Methods: We measured airway responsiveness to methacholine using dosimeter and tidal breathing methods in 30 individuals with suspected asthma. Concentration-response curves were characterized by their PC_{20} (sensitivity), slope (reactivity), and, if possible, level of plateau.

Results: Dosimeter PC_{20} values were significantly higher than tidal breathing values (geometric mean, 8.9 and 5.2 mg/mL, respectively); the mean difference in PC_{20} values obtained using each method was 0.78 doubling concentrations ($P = .01$). The mean slopes were 22.7%/log mg/mL using the tidal breathing method and 24.9%/log mg/mL using the dosimeter method; the mean difference in the slopes obtained using each method was −2.17%/log mg/mL ($P = .18$). In 10 individuals who showed a plateau with the 2 methacholine challenge tests, the mean level of plateau was 19.8% using the tidal breathing method and 19.5% using the dosimeter method; the mean difference in the plateau values obtained with each method was 0.3% ($P = .87$).

Conclusions: Although the tidal breathing method produces methacholine PC_{20} values significantly lower than the dosimeter method, both methods provide similar values for slope and level of plateau. These results suggest that the technical factors that affect methacholine sensitivity and the shape of the curve are different.

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INTRODUCTION

It is now generally accepted that asthma is characterized by chronic inflammation of the airways, bronchial hyperresponsiveness, and paroxysmal attacks of wheezing.[1] Clinically and for research purposes, methacholine challenge has been widely used for the detection and quantitation of airway responsiveness.[2] The response to this bronchoconstrictor agent is called *sensitivity* and is commonly expressed as the provocation concentration (PC_{20}) or provocation dose (PD_{20}) that caused a decrease in forced expiratory volume in 1 second (FEV_1) of 20%. A decreased PC_{20} or PD_{20} value then indicates the presence of hypersensitivity to the bronchoconstrictor agent, and, in the literature, this term is often regarded as being synonymous with the term *hyperresponsiveness*. The specificity and sensitivity of PC_{20} in the objective evaluation of airway hypersensitivity in asthmatic patients compared with healthy individuals have been described elsewhere.[2]

However, airway hyperresponsiveness to a provoking stimulus should be defined as the profile of the tendency of the airways to narrow across a wide range of intensities of that stimulus.[3] The severity of the response is reflected by the shape of the dose-response curve, and previous studies[4,5] have focused on the importance of characterizing the entire methacholine dose-response curve not only by sensitivity but also by reactivity (slope of the dose-response curve) and the maximal airway narrowing response value (plateau). A steepening of the slope should be referred to as *hyperreactivity*,[6] and this term should not be regarded as being synonymous with *hypersensitivity*. Furthermore, if sigmoid dose-response curves can be recorded safely, the presence and level of a maximal response plateau provide relevant information on the potential severity of airways obstruction.[4,5,7–10] A maximal response plateau is a feature of nonasthmatic individuals,[4,7,8] whereas it is infrequently detected in patients with mild asthma. In addition, it has been reported that the mechanisms that modulate sensitivity and the plateau level are at least partially different.[11,12] Thus, identification of the effect of some therapeutic interventions[13] on the level of plateau may add relevant information to that obtained from determination of the PC_{20}. For this reason, previous investigations[4,5,7–10] have focused on the importance of measuring variables of the entire dose-response curve (ie, the reactivity and the plateau value) in research studies, because the distinction of these components of hyperresponsiveness may have implications for the diagnosis and treatment of asthma.[13,14]

At present, 2 methods of inhalation challenge have been used. The first, introduced by Cockcroft and colleagues,[2] consists of continuous generation of aerosol and inhalation by quiet tidal breathing at a spontaneous frequency for 2 min-

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utes. The second, proposed by Chai and coworkers,[15] consists of intermittent aerosolization with use of a breath-activated dosimeter for 5 inhalations. It has been suggested in several studies[16–18] that the 2 methods give comparable results for methacholine sensitivity, and, for this reason, both methods of methacholine challenge testing are recommended by American Thoracic Society[19] and European Respiratory Society[20] guidelines. However, recent data suggest that the tidal breathing method may produce methacholine PC_{20} values significantly lower than the dosimeter method.[21] Furthermore, in these studies, airway responsiveness was expressed as the PC_{20}, and it remains uncertain whether the challenge method may have some effect on the shape of the dose-response curve to methacholine. Thus, the aim of this study was to investigate differences in PC_{20}, slope, and maximal response plateau between concentration-response curves to inhaled methacholine obtained using the tidal breathing and dosimeter methods in adults with suspected asthma.

METHODS

Participants

Thirty individuals undergoing methacholine challenge for clinical evaluation of suspected asthma were recruited from the outpatient allergy clinic at Hospital Universitario Dr Peset. At the time of the study, FEV_1 was at least 80% of predicted. All the patients were lifelong nonsmokers, and none had a history of chronic bronchitis, emphysema, or respiratory tract infections during the 4 weeks before the study. Current smokers, pregnant women, and patients with significant renal, hepatic, or cardiovascular disease were excluded. The study protocol was approved by the local ethics committee, and written informed consent was obtained from all the participants.

Study Design

This was an open, randomized, crossover study. Patients attended the laboratory on 3 days, at the same time each day. Short-acting inhaled β_2-agonists (n = 16) were withheld for at least 6 hours before each challenge, long-acting inhaled β_2-agonists (n = 3) for at least 24 hours, inhaled corticosteroids (n = 9) for at least 2 weeks, and oral antihistamines (n = 11) for at least 72 hours. Theophylline, oral corticosteroids, leukotriene receptor antagonists, sodium cromoglycate, nedocromil sodium, and anticholinergic bronchodilators were not used by any patients during the month before being studied. Use of nasal topical corticosteroids (n = 7) was continued at the same dose. On the first day, all the patients were evaluated for suitability, and spirometry was performed. On each of the next 2 visits (at least 1 day but not >5 days apart), spirometry and methacholine challenges using either the tidal breathing or the dosimeter method were performed. The methacholine challenges using each method were conducted on separate days, with the order of challenge randomized. For each test, the baseline FEV_1 was required to be within 10% of the initial baseline FEV_1 and at least 80% of predicted,[22] because patients with these characteristics are representative of the type of patients in whom methacholine challenge testing may be clinically useful.

Pulmonary Function

Lung function (flow-volume curves) was measured using a calibrated pneumotachograph (Jaeger MasterScope; Erich Jaeger GmbH, Würzburg, Germany) according to standardized guidelines.[23] Baseline FEV_1 and forced vital capacity levels were measured until 3 reproducible recordings differing by less than 5% were obtained. Maneuvers were accepted as technically satisfactory if the back-extrapolated volume was less than 150 mL or 5% of forced vital capacity and if the expiratory time was at least 6 seconds. The highest values were used for analyses. Reference values were those of the European Community for Coal and Steel.[22]

Methacholine Challenge Procedures

Methacholine challenge was performed according to the protocol of Cockcroft et al.[2] Following baseline spirometry, FEV_1 was measured after inhalation of isotonic sodium chloride for 2 minutes, followed by doubling concentrations of methacholine (Sigma-Aldrich Corp, St Louis, MO) solutions in isotonic sodium chloride in concentrations of 0.39 to 200 mg/mL. Aerosols were delivered by means of a nebulizer (model 1720; Hudson, Temecula, CA) with 2 mL of test solution in the container and a mean ± SD delivery rate of 0.13 ± 0.02 mL/min. The nebulizer delivers particles with an aerodynamic mass median diameter of 2.2 μm. The mean ± SD output of the nebulizer (the amount delivered to the mouth) was determined by weighing the nebulizer containing 2 mL of isotonic sodium chloride solution before and after 2 minutes of activation, with a technician simulating the test, on 3 occasions. The nebulizer was connected directly to a mouthpiece, a nose clip was worn, and the aerosol was inhaled through the mouth by means of tidal breathing for 2 minutes. A single determination of FEV_1 was taken 60 to 90 seconds after inhalation of each concentration[24] unless the forced expiratory maneuver was judged to be technically unsatisfactory. The test was interrupted when FEV_1 decreased by more than 40% from its postsaline value or when the highest concentration of methacholine had been administered.

The other methacholine challenge procedure was performed using a jet nebulizer attached to a breath-activated dosimeter[15] (model MB3; Mefar, Brescia, Italy) at a nebulization time of 1 second and a pause time of 6 seconds. The nebulizer delivers particles with an aerodynamic mass median diameter of 3.5 to 4.0 μm at an output of 10 μL per breath. The nebulizer output was checked by weighing the nebulizer containing 2 mL of isotonic sodium chloride solution before and after 10 actuations, with a technician simulating the test by inhaling from the nebulizer, on 3 occasions. The 5-breath dosimeter methacholine challenge was performed using identical methacholine solutions (0.39 to 200 mg/mL), starting concentrations, FEV_1 timing, etc, as the tidal breathing method. Only the inhalation method was dif-