Effect of pH on bronchial response to inhaled histamine

DW COCKCROFT, BA BERSCHIED

From the Division of Pulmonary Medicine, University Hospital, Saskatoon, Saskatchewan, Canada

ABSTRACT In order to investigate the effect of pH on bronchial responsiveness to inhaled histamine, 15 subjects with non-specific bronchial hyperreactivity performed two histamine inhalation tests, one with unbuffered, and the other with buffered histamine acid phosphate solutions. The unbuffered histamine solutions were prepared with 0.9% sterile saline and had a pH range from 4.3 to 7.3, while the buffered histamine solutions were prepared with a phosphate buffer and had a pH range of 6.5 to 7.4. The two histamine inhalation tests were similar in all other regards. The geometric mean histamine provocation concentration required to produce a 20% reduction in FEV₁ (PC₂₀) was significantly lower for the unbuffered histamine (1.33 mg/ml) than for the buffered histamine (1.67 mg/ml), $p < 0.05$. The two PC₂₀s differed by less than one doubling dilution, the range of reproducibility of the test, in 12 of the 15 subjects. The pH effect was only noted when the pH of the histamine solutions was below five (histamine concentrations from one to eight mg/ml). We conclude that the acid pH of higher concentrations of histamine acid phosphate solutions has a slight but significant enhancing effect on the bronchial responsiveness to inhaled histamine.

Inhaled histamine induces bronchoconstriction in most subjects with bronchial asthma,¹⁻⁵ in some with other respiratory diseases^{2,4,6} and in a few normal subjects.⁵ The mechanism of histamine's action and the mechanism underlying such non-specific bronchial hyperreactivity are not entirely clear. One feature of histamine which may affect bronchial response is its acidity. In this investigation, the effect of acid pH on bronchial responsiveness to inhaled histamine was examined by comparing bronchoconstriction induced by buffered and unbuffered inhaled histamine in 15 stable subjects with known non-specific bronchial hyperreactivity.

Methods

HISTAMINE INHALATION TEST

Histamine inhalation tests were performed as described by Cockcroft *et al*⁵ and Juniper *et al*.⁷ Solutions were nebulised with a Wright nebuliser operated with an airflow rate of seven litres per minute, delivering an aerosol with an output of 0.130 ml/min and a mass median diameter particle size of one μ m. The nose was clipped, and inhalations were made by quiet breathing through the

mouth for two minutes. After a control inhalation of the diluent, doubling concentrations of histamine acid phosphate from 0.03 to 8.0 mg/ml were inhaled at five-minute intervals. The forced expiratory volume in one second (FEV₁) was measured in triplicate with a Godart nine-litre water spirometer prior to the inhalations and was repeated 30 and 90 seconds after each inhalation. The test was continued until the FEV₁ had fallen by 20% or more, or until the top concentration of histamine had been administered. The results were expressed as the provocation concentration of histamine producing a 20% reduction in FEV₁ (PC₂₀).

Unbuffered histamine acid phosphate solutions were prepared with 0.9% sterile saline (pH 7.3) and the buffered histamine solutions were prepared with a sterile isotonic phosphate buffer (NaH₂PO₄ H₂O 1.808 grams, Na₂HPO₄ 7.576 grams, and NaCl 4.4 grams in 1000 ml H₂O) with a pH of 7.4. The pH of each solution was determined using a Corning pH and blood gas analyser (Model no 165).

SUBJECTS

Fifteen subjects with known bronchial hyperresponsiveness to unbuffered inhaled histamine (PC₂₀ $\leq$ 8.0 mg/ml) were selected from the Respiratory Clinic at the University Hospital in Saskatoon. These subjects were selected to have a wide range of

Address for reprint requests: Dr DW Cockcroft, Pulmonary Medicine, University Hospital, Saskatoon, Saskatchewan, Canada S7N 0X0.

bronchial responsiveness to inhaled histamine. Twelve of these subjects had documented asthma with episodic wheezing and dyspnoea and clinical response to inhaled bronchodilator. The remaining three had mild histamine responsiveness associated with allergic rhinitis or as a mild variation of normal.

STUDY DESIGN

Studies were done on two consecutive days at the same time of day. Factors known to influence non-specific bronchial reactivity were controlled. Subjects were not smoking, had not had symptoms of a respiratory infection for at least four weeks, and had not been exposed to relevant allergens for at least four weeks. Baseline FEV₁ was stable within 10% on the two test days. Inhaled bronchodilators were withheld for six hours before the tests, oral theophylline preparations for 12 hours before the tests, and steroids were continued in the same dose. No subjects were using oral adrenergic bronchodilators, sodium cromoglycate or antihistamines.

On one day, the histamine inhalation test was done using the buffered solutions and on the other day using the unbuffered solutions. The tests were performed in random order.

ANALYSES

Logarithmic transformation of histamine PC20 was used in the analysis. Analyses were performed using the paired *t* test⁸ and the method of least squares linear regression.⁸

Results

The pH of the unbuffered and buffered histamine solutions is shown in the table. The pH ranged from 4.3 (8 mg/ml) to 7.3 (diluent) for the unbuffered solutions and from 6.5 (8 mg/ml) to 7.4 (diluent) for the buffered solutions.

The geometric mean buffered and unbuffered PC20s were compared with the paired *t* test. The geometric mean buffered PC20 was 1.67 mg/ml

(geometric standard deviation 5.5) and the geometric mean unbuffered PC20 was 1.33 mg/ml (geometric standard deviation 4.68). There was a significant difference between these two means (*p* < 0.05).

The buffered and unbuffered PC20s were plotted graphically in the figure. Ten of the subjects had both results very close to the line of identity, and 12 of the 15 subjects showed a difference less than one doubling dilution between the two tests. The remaining three subjects had a buffered PC20 that was ≥ twice the unbuffered PC20. These significant deviations from the line of identity only occurred with concentrations of histamine ≥ 1.0 mg/ml—that is, those with a pH less than 5.0. Below a concentration of 1.0 mg/ml all points were near the line of identity.

The regression line for this plot was as follows: Log₁₀ (unbuffered PC20) = 0.9 × Log₁₀ (buffered PC20) – 0.07 (*r* = 0.98, *s* = 7.03).

Figure Logarithmic plot of unbuffered histamine PC20 and buffered histamine PC20. The PC20 in mg/ml determined with unbuffered histamine solutions (0.9% NaCl diluent) is on the vertical axis and the PC20 in mg/ml using buffered solutions (phosphate buffer diluent) is on the horizontal axis. The heavy line is the line of identity and the lighter lines represent one doubling dilution either side of the line of identity—the range of reproducibility of the test.^{7 9 10}

Discussion

The results demonstrated that the acid pH of histamine solutions had a slight but significant effect in increasing the histamine-induced bronchoconstriction. This effect was evident when mean buffered

Table pH of histamine solutions

Histamine acid phosphate concentration (mg/ml)	Unbuffered (0.9% NaCl diluent)	Buffered (phosphate buffer diluent)
Diluent	7.3	7.4
0.031	6.4	7.4
0.062	6.3	7.4
0.125	5.7	7.3
0.25	5.4	7.3
0.50	5.3	7.2
1.0	4.8	7.1
2.0	4.7	6.9
4.0	4.4	6.7
8.0	4.3	6.5

PC20 and unbuffered PC20 were compared; the overall slight difference, 1.67 and 1.33 mg/ml respectively, was significant $p < 0.05$). However when individual data were examined, only three subjects had results outside the limits of reproducibility of the test. The histamine inhalation test performed in this manner is reproducible to within one doubling dilution.^{7 9 10} All three subjects outside this limit showed a greater bronchoconstrictor effect with the more acid unbuffered histamine.

The test differences appeared to relate to the pH of the solutions. There was little difference when the histamine concentrations were under 1 mg/ml (figure)—that is, where unbuffered solutions had pH above 5.0 (table). The acid pH (< 5.0) of the higher histamine concentrations, 1.0 to 8.0 mg/ml, was associated with slight enhanced effect in five of 10 (figure), three of these five beyond the limits of reproducibility. There was no difference between the two tests in the remaining five responders to concentrations between 1 and 8 mg/ml. Thus this pH-enhanced bronchoconstriction was suggested in about 50% of subjects responding to histamine in concentrations of 1.0 to 8.0 mg/ml and none of the subjects responding to concentrations below 1.0 mg/ml.

The mechanism of action of inhaled histamine is not completely understood. Inhaled histamine is currently thought to act in part by stimulating subepithelial irritant receptors and inducing reflex vagal bronchoconstriction^{11 12} and in part by a direct bronchoconstrictor effect on the bronchial smooth muscle.^{12 13} The low pH of the higher histamine concentrations may contribute to bronchoconstriction by increasing irritant receptor stimulation. Aerosols of citric acid have been shown to produce bronchoconstriction;^{6 11} an effect which has been attributed to the low pH.⁶ This effect can be inhibited by atropine suggesting that vagal pathways, probably stimulation of subepithelial irritant receptors, are relevant in acid-induced bronchospasm.^{6 11}

The importance of this slight pH effect on the performance of inhalation tests depends on the purpose of the test. Since the effect is small and almost certainly nonspecific, it is probably not an important consideration in the design of bronchial provocation tests used clinically to measure nonspecific bronchial reactivity. The histamine inhalation test described here is considered normal if the unbuffered histamine PC20 is greater than 8.0 mg/ml.⁵ The results of the current investigation suggest that if buffered histamine were routinely used, the lower limit of normal would need increasing, perhaps to 16 mg/ml. This would require use of a 16 mg/ml histamine solution which has been shown to produce

an unacceptable incidence of systemic side-effects for routine clinical use.⁷ On the other hand, if bronchial provocation tests are being designed to investigate the mechanism of action of a bronchoconstrictor agent, or if the tests are designed to demonstrate "specific" bronchial hyperreactivity—for example allergen inhalation tests—then clearly this non-specific pH effect should be considered. In such circumstances, solutions for inhalation should be buffered so that the pH is above 5.0 and preferably above 6.0. In any event, it is important that inhalation tests performed for any reason should be well standardised,¹⁴⁻¹⁷ and pH is one factor which must be considered in such standardisation.¹⁷

In conclusion, the acid pH, below 5.0, of histamine acid phosphate solutions ≥ 1.0 mg/ml plays a small but significant role in enhancing the bronchoconstrictor effect of inhaled histamine in some subjects. The effect is probably non-specific and probably involves stimulation of afferent vagal irritant receptors. Although of minimal importance in identification of nonspecific bronchial hyperreactivity, this effect is important to consider when examining mechanisms of bronchoconstriction or when designing specific (allergen) inhalation tests.

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References

- Curry JJ. The action of histamine on the respiratory tract in normal and asthmatic subjects. *J Clin Invest* 1946;**25**:785-91.
- Townley RG, Dennis M, Itkin IH. Comparative action of acetyl-beta-methyl-choline, histamine and pollen antigens in subjects with hayfever and patients with bronchial asthma. *J Allergy* 1965;**36**:121-37.
- Herxheimer H. Provocation of bronchial obstruction in bronchial asthma by dilute histamine aerosols. *Acta Allerg* 1972;**27**:212-8.
- Laitinen LAI. Histamine and methacholine challenge in the testing of bronchial reactivity. *Scand J Respir Dis* 1974; Suppl **86**:1-48.
- Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;**7**:235-43.
- Empey DW, Laitinen LA, Jacobs L, Gold WM, Nadel JA. Mechanism of bronchial hyperreactivity in normal subjects after upper respiratory infection. *Am Rev Respir Dis* 1976;**113**:131-9.
- Juniper EF, Frith PA, Dunnett C, Cockcroft DW, Hargreave FE. Reproducibility and comparison of responses to inhaled histamine and metacholine. *Thorax* 1978; **33**:705-10.
- Steel RDG, Torrie JH. *Principles and procedures of statistics with special reference to the biological sciences.*

- New York: McGraw-Hill, 1960;78:161-93.
- ⁹ Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Protective effect of drugs on histamine-induced asthma. *Thorax* 1977;32:429-37.
 - ¹⁰ Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in non-allergic bronchial reactivity. *Clin Allergy* 1977;7:503-513.
 - ¹¹ Simonsson BG, Jacobs FM, Nadel JA. Role of autonomic nervous system and the cough reflex in the increased responsiveness of airways in patients with obstructive airways disease. *J Clin Invest* 1967;46:1812-8.
 - ¹² Sellick H, Widdicombe J. Stimulation of lung irritant receptors by cigarette smoke, carbon dust, and histamine aerosol. *J Appl Physiol* 1971;31:15-19.
 - ¹³ Casterline CL, Evans R III. Further studies on the mechanism of human histamine-induced asthma. *J Allergy Clin Immunol* 1977;59:420-4.
 - ¹⁴ Chai H, Farr RS, Froelich LA *et al.* Standardization of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975;56:323-7.
 - ¹⁵ Orehek J, Gayraud P. Les tests de provocation bronchique non-spécifiques dans l'asthme. *Bull Europ Physiopathol Respir* 1976;12:565-98.
 - ¹⁶ Dolovich M, Obminski G, Cockcroft DW, Hargreave FE, Newhouse MT. Standardization of inhalation tests: influence of method of aerosol generation and inhalation. *J Allergy Clin Immunol* 1979;63:165.
 - ¹⁷ Cropp GJA, Bernstein IL, Boushey HA, Hyde RW, Rosenthal RR, Spector SL, Townley RG. Guidelines for bronchial inhalation challenges with pharmacologic and antigenic agents. *American Thoracic Society News* 1980;6:11-19.