

Reproducibility and comparison of responses to inhaled histamine and methacholine

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The efficiency of a standardised inhalation test procedure was studied by examining the reproducibility of responses to histamine and methacholine. In addition, the responses to the two agents were compared. Each set of duplicate tests was carried out on a separate day within one week, and all factors known or presumed to influence responses were carefully controlled. The results were expressed as the provocative concentration of the agent causing a 20% fall in forced expired volume in one second (PC_{20}). Responses to histamine and methacholine were highly reproducible (coefficients of determination [r^2] = 0.994 and 0.990 respectively). Responsiveness to histamine correlated closely with responsiveness to methacholine ($r^2 = 0.85$). There was a small but significant cumulative dose effect with methacholine ($P < 0.01$) but not with histamine. Side effects of throat irritation, flushing, and headache were more frequent with histamine than methacholine, and were dose-related. The high level of reproducibility indicates the efficiency of the test procedure. The similar severity of effects by agents with different mechanisms of action suggests that the primary cause of non-specific bronchial hyperreactivity lies at the level of bronchial smooth muscle.

Non-specific bronchial reactivity is the term used to indicate the responsiveness of the airways to chemical mediators, such as histamine, acetylcholine, and prostaglandin $F_{2\alpha}$, or to the synthetic analogues of acetylcholine, such as methacholine and carbachol. Its measurement is becoming increasingly important in clinical practice, epidemiology, and research.

Non-specific bronchial reactivity is measured by inhalation tests, usually with histamine or methacholine. In the past different methods have been used, making critical analysis of results difficult. Recently Chai *et al* (1975) and Orehek and Gayraud (1976) have emphasised the importance of the standardisation of tests. Standardisation requires consideration of the many different factors that will influence the results.

In this study we have standardised a simple inhalation test using histamine and methacholine. We have investigated the efficiency of the method by examining the reproducibility of responses over a short period. In addition we have compared the responsiveness to each agent and investigated the

possibility of cumulative dose-effects and recorded side effects.

Subjects and methods

SUBJECTS

The patients were adults with asthma attending the regional chest and allergy unit. All had episodic dyspnoea or wheezing and documented variation in forced expired volume in one second (FEV_1) of more than 20%, either spontaneously or after medication. None had features of any other respiratory disorder.

Normal adults were volunteers from hospital staff. They had no present or past symptoms of rhinitis, asthma, or other respiratory disorder. They were non-smokers, non-atopic, and had normal spirometry. Non-atopic implied that they had no early wheal and flare responses to prick tests with extracts of *Alternaria*, *Cladosporium*, *Aspergillus fumigatus*, cat, dog, feathers, horse, housedust, *Dermatophagoides farinae*, egg white, milk, nuts, shellfish, tree pollen, grass pollen, and

ragweed pollen. Informed signed consent was obtained.

INHALATION TESTS

Inhalation tests were carried out by the method described for histamine by Cockcroft *et al* (1977a). Aerosols of physiological saline, histamine acid phosphate or methacholine were delivered into a face mask from a Wright nebuliser with an output of 0.13 ml/min and aerosol particles of 1.3 μm mass median diameter and 2.11 μm geometric standard deviation. A clip was placed on the nose and each aerosol was inhaled through the mouth by tidal breathing for 2 min. Physiological saline was inhaled first, followed at 5-min intervals by histamine or methacholine in twofold increasing concentrations from 0.03 to 16 mg/ml. The histamine solutions were made up as needed at intervals up to three months; methacholine solutions were renewed every two weeks.

The FEV₁ was measured using a Collins 13.5 l water spirometer. Initially the manoeuvre was repeated until three reproducible values were obtained. After each inhalation it was measured at 0.5 and 1.5 min and, if necessary, at 3 min and subsequent intervals of 2 min to obtain the lowest post-inhalation FEV₁. The FEV₁ was measured only once on these occasions unless the measurement was technically unsatisfactory.

Inhalations were continued until the FEV₁ had fallen by 20% or more. The change in FEV₁ was calculated from the lowest post-saline value. Responses were expressed in terms of the provocative concentration of histamine or methacholine producing a 20% fall in FEV₁ (PC₂₀). This was obtained from the log dose-response curve.

STUDY DESIGN

All comparison tests were performed single-blind at the same time of day on separate days within one week. In each subject the baseline FEV₁ was required to be more than 70% of predicted and within $\pm 5\%$ of values on the previous test day. Subjects were included only if they had had no respiratory infection or vaccination, no exposure to relevant allergens, and no salicylate ingestion for six weeks. Inhaled beta-adrenergic agonists and sodium cromoglycate were withheld for eight hours, ingested bronchodilators for 24 hours, and antihistamines for 48 hours before each study. Prednisone or beclomethasone dipropionate aerosol were continued in the same dose.

The short-term reproducibility of responses to histamine and to methacholine were investigated by performing two identical inhalation tests with each agent. Fourteen subjects (11 asthmatic, three

normal) were tested with histamine, and another group of 13 subjects (11 asthmatic, two normal) were tested with methacholine.

Response to histamine and methacholine were compared in 33 asthmatic and 14 normal subjects by performing one identical test with each agent. Twenty-three of these subjects, with a PC₂₀ of 1 mg/ml or above, completed a questionnaire regarding side effects after each test. The possibility of a cumulative dose effect produced by the method was examined for histamine in 15 subjects (14 asthmatics, one normal) and for methacholine in a different group of 10 subjects (eight asthmatic, two normal). In each subject one test was carried out using the dose-response method. A second test was performed giving only the final concentration inhaled in the first test. The PC₂₀ was obtained for the first test as previously described; for the second test it was calculated by the formula:

$$\frac{\text{concentration of agent inhaled} \times 20}{\% \text{ fall in FEV}_1}$$

ANALYSIS

Values of PC₂₀ were compared using linear regression analysis and Student's paired *t* test.

Results

There was a high degree of reproducibility of responses to histamine; the coefficient of determination (r^2) was 0.994, (fig 1a). Responses to methacholine were also reproducible ($r^2=0.990$) (fig 1b).

There was a close correlation between the severity of responses to histamine and methacholine ($r^2=0.85$) (fig 2).

Inhalation of at least three doubling concentrations of methacholine at intervals of 5 min produced a small cumulative effect in every subject (fig 3a). For the group this was a significant effect ($P<0.01$). By contrast, no cumulative effect was seen with histamine (fig 3b).

Dyspnoea, chest tightness, or wheezing occurred with equal frequency after both agents in association with the fall in FEV₁. Irritation of the throat and cough occurred alone or in association with headache or flushing more often with histamine than with methacholine; they were dose related (fig 4). Among those subjects with "irritant" and systemic symptoms, seven had a fall in FEV₁ of less than 20% after inhalation of histamine 8 mg/ml. These seven were unable to tolerate histamine at 16 mg/ml but were able to tolerate methacholine up to 16 mg/ml, allowing falls in FEV₁ of greater than 20% to occur.

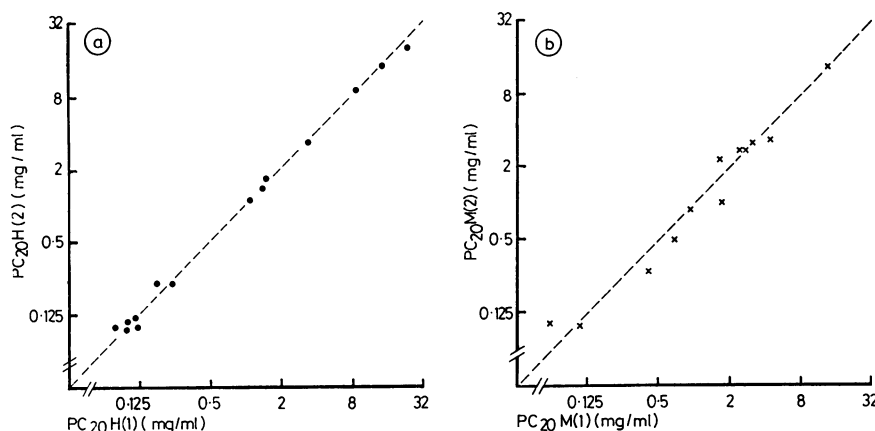


Fig 1 Reproducibility of responses to inhaled histamine (a) and methacholine (b). $PC_{20}H(1)$ is PC_{20} from first test with histamine; $PC_{20}H(2)$ is PC_{20} from second; and $PC_{20}M(1)$ and $PC_{20}M(2)$ are corresponding symbols for methacholine. Dashed lines are lines of identity. Coefficient of determination = 0.994 for histamine and 0.990 for methacholine.

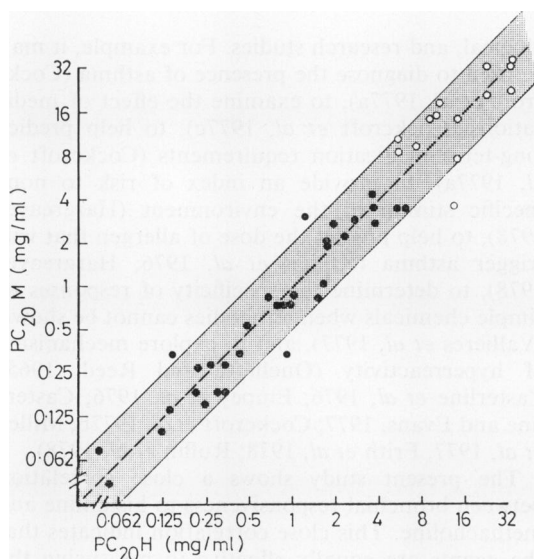


Fig 2 Comparison of responses to inhaled histamine and methacholine. Open circles represent normal subjects ($n=14$) and closed circles asthmatics ($n=33$). Cross-hatching indicates region of single twofold concentration difference. Dashed line is line of identity. Coefficient of determination = 0.85.

Discussion

In this study we have shown that the inhalation test method is capable of inducing responses to histamine and methacholine that are highly reproducible in severity. This is a result of the careful

standardisation of the method, which includes consistent generation of aerosol, inhalation by tidal breathing, the use of FEV_1 as a measure of response, and the careful control of other factors known or presumed to influence the response. Nebuliser output and particle size are both important in relation to the consistent generation of aerosol. Nebuliser output varies considerably between nebulisers and is important to control (Mercer, 1973); particle size also varies between nebulisers, although between $1.3 \mu m$ and $3.6 \mu m$ it does not influence the response (Dolovich *et al*, 1978). The method of inhalation influences the dose reaching the lung and the pattern of deposition in the lung (Pavia *et al*, 1977). In the present study tidal breathing was used in preference to vital capacity inspirations because the latter influence lung mechanics (Gayrard *et al*, 1975; Fish *et al*, 1977) and produce more variability in lung dose and deposition pattern unless the speed of inspiration is carefully controlled (Pavia *et al*, 1977). Other "non-technical" factors that may influence the response and which were controlled include time of day (deVries *et al*, 1962), degree of initial airflow obstruction (Benson, 1975), respiratory infection (Empey *et al*, 1976) vaccination (Ouellette and Read, 1965), allergen exposure (Altounyan, 1971; Cockcroft *et al*, 1977b), smoking, and medications (Chai *et al*, 1975; Cockcroft *et al*, 1977c). Variation in the influence of these factors may alter bronchial responsiveness over longer periods.

The inhalation test method is simple and portable, and is therefore suitable for clinical, epidemi-

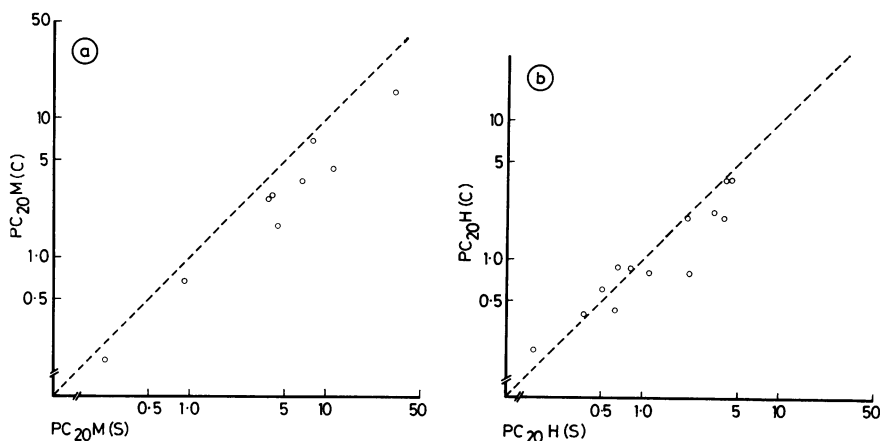


Fig 3 Cumulative dose effect of inhaled methacholine (a) and histamine (b). $PC_{20}H$ (c) is PC_{20} calculated from standard dose-response inhalation test with histamine; $PC_{20}H$ (S) is PC_{20} calculated from a single dose inhalation test with histamine; Same symbols are used for $PC_{20}M$. Dashed line is line of identity. Coefficient of determination = 0.79 for histamine and 0.94 for methacholine.

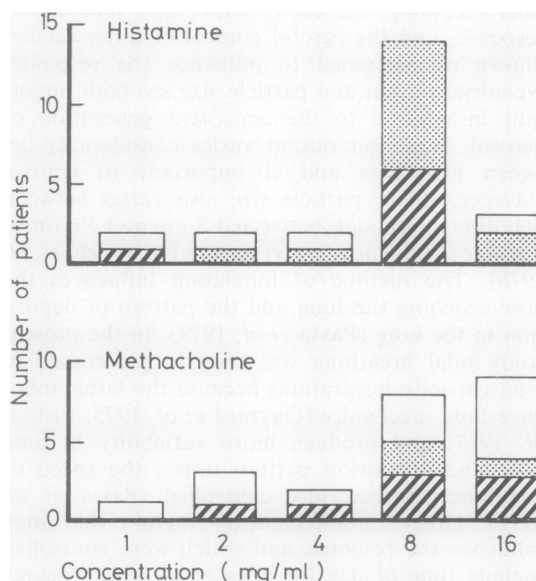


Fig 4 "Irritant" and systemic side effects from higher doses of inhaled histamine and methacholine. Concentration refers to final concentration tolerated by virtue either of 20% fall in FEV_1 or of side effects. □ Subjects with no symptoms; ▨ Subjects with throat irritation and coughing alone; ▩ Subjects with throat irritation and coughing, and headache or flushing. Mild irritant and systemic symptoms were more common with histamine.

ological, and research studies. For example, it may be used to diagnose the presence of asthma (Cockcroft *et al*, 1977a), to examine the effect of medications (Cockcroft *et al*, 1977c), to help predict long-term medication requirements (Cockcroft *et al*, 1977a), to provide an index of risk to non-specific stimuli in the environment (Hargreave, 1978), to help predict the dose of allergen that will trigger asthma (Killian *et al*, 1976; Hargreave, 1978), to determine the specificity of responses to simple chemicals when antibodies cannot be shown (Vallières *et al*, 1977), and to explore mechanisms of hyperreactivity (Ouellette and Reed, 1965; Casterline *et al*, 1976; Empey *et al*, 1976; Casterline and Evans, 1977; Cockcroft *et al*, 1977b; Miller *et al*, 1977, Frith *et al*, 1978; Ruffin *et al*, 1978).

The present study shows a close correlation between bronchial responsiveness to histamine and methacholine. This close correlation indicates that the agents are equally effective in measuring the level of non-specific bronchial reactivity. The similarity in responsiveness also has implications in understanding the cause of the non-specific bronchial hyperreactivity of asthma, which at present remains unknown. One hypothesis is that there is a heightened sensitivity of irritant receptors and a consequent exaggerated reflex cholinergic bronchoconstriction (Nadel, 1977). The results of the present study suggest that this is not the only abnormality. Studies on the mechanism of action of inhaled histamine in man indicate that it acts partly through reflex cholinergic pathways and partly by direct effects on histamine H_1 receptors

(Casterline *et al*, 1976; Casterline and Evans, 1977; Ruffin *et al*, 1978) and H₂ receptors (Frith *et al*, 1978). By contrast methacholine is considered to have no effect on irritant receptors and to exert its effect through cholinergic receptors on smooth muscle (Goodman and Gilman, 1975; Miller *et al*, 1976). In the present study the similar severity of effects by agents with different mechanisms of action suggests that the primary cause of the hyper-reactivity lies at the level of smooth muscle. Such an alternative hypothesis has already been suggested by Antonissen *et al* (1977) and Miller *et al* (1978).

In the present study inhalation of methacholine at 5-min intervals produced a cumulative effect, which was not seen with histamine. Histamine, however, produced more "irritant" and systemic side effects at higher doses. These differences between the effects of histamine and methacholine, which have been observed by others (Orehek and Gayrard, 1976), need to be taken into account when considering which agent to use in studies of non-specific reactivity. Other possible differences between the agents include the shelf life and the degree of change in reactivity after allergen exposure. The shelf life is not accurately known, but is probably longer for histamine (years) than methacholine (weeks) (Windholz, 1976). Changes in responsiveness to histamine after allergen-induced asthma are greater than changes in responsiveness to methacholine (Cockcroft *et al*, 1977b). The present study shows, however, that when factors that modify responses are controlled, either agent may be used with equal efficiency to show the level of non-specific bronchial reactivity.

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