Refractory period following bronchoconstriction provoked by histamine in asthmatic subjects

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ABSTRACT   To determine whether refractoriness to histamine induced bronchoconstriction occurs, 20 asthmatic subjects aged 19–50 years were tested. Subjects underwent two histamine challenge tests (1 and 2) on the same day, the second one being given 45–60 minutes after the first, once the FEV_1 after test 1 had returned spontaneously to within 90% of baseline. A further "control" histamine challenge test was carried out on a different day at the same time (± 2 hours) as test 1. Bronchial responsiveness was recorded as the cumulative dose (μg) of histamine provoking a 20% fall in FEV_1 (PD_{20}), and the ratio PD_{20} test 2:PD_{20} test 1 was used to assess refractoriness. The median value of this ratio (2·20) was significantly greater than 1 (p = 0·003), indicating refractoriness at the time of test 2. By contrast the median ratio PD_{20} control: PD_{20} test 1 of 1·03 was not significantly different from 1. Refractoriness could not be accounted for by failure to regain the initial baseline FEV_1, though such failure may have exaggerated the effect. An increase in PD_{20} with the second test was observed uniformly in subjects with moderate or high initial PD_{20} values but not in those with low values. This suggests that there may be a PD_{20} threshold of the order 25–100 μg for refractoriness to occur. Refractoriness could exert an important confounding effect in investigations in which repeated histamine tests are carried out at short intervals.

Introduction

Controlled provocation of bronchoconstriction by chemical and physical stimuli is widely used in the investigation of asthma. Immediate refractoriness to further bronchoconstriction has been shown to occur when the initial constrictive episode is provoked by stimuli such as exercise[1]; hyperventilation[2]; or the inhalation of ultrasonically nebulised distilled water,[3] leukotriene D4,[4] and platelet activating factor.[5] With methacholine the long duration of action makes the investigation of refractoriness difficult. Refractoriness after methacholine provoked bronchoconstriction has, nevertheless, been reported by some investigators though not others.[6][7]

Histamine has been the subject of more detailed study, though data are still limited and conflicting. In 1980 Schoeffel *et al* found progressively smaller mean decrements in peak expiratory flow (38%, 34%, and 29%) in 16 asthmatic subjects after inhalation of identical doses of histamine at 40 minute intervals.[8] This initial hint of refractoriness after histamine

challenge tests has been supported by recent reports—those of Manning and colleagues,[9][10] who retested 11 and eight subjects respectively after 60 minutes using the continuous (Wright) nebulisation method, and of Magnussen and colleagues, who retested nine subjects after 60 minutes using airway resistance to measure bronchial responsiveness.[6] Controversy has arisen, however, because other studies, using a similar range of methods, have failed to show significant decreases in bronchial responsiveness with closely repeated histamine tests.[11–14] We present the results of a study in 20 adults with asthma.

Methods

SUBJECTS
Twenty subjects (9 male, 11 female) with symptomatic asthma and reversible airway obstruction were recruited from our clinic and student populations. They were not told the purpose of the study. The age range was 19–50 (median 25) years. All were non-smokers and 18 were atopic. Each subject was required to have a forced expiratory volume in one second (FEV_1) on entry of at least 60% of the predicted value. Subjects who had had a respiratory tract infection or had received antihistamines,

cromoglycate, nedocromil, or oral corticosteroids within the preceding six weeks were excluded. They had to avoid oral bronchodilators and aspirin for 48 hours before each histamine test, and inhaled bronchodilators, ipratropium, and corticosteroids for 24 hours.

MEASUREMENT OF VENTILATORY FUNCTION

FEV_1 was measured as the mean of three technically satisfactory measurements with a bellows spirometer (Vitalograph Ltd, Buckingham). Baseline FEV_1 was taken as the mean of three measurements 10 and five minutes before and at the start of the histamine aerosol challenge (that is, the mean of nine recordings).

HISTAMINE SOLUTIONS

Sequential doubling dilutions of histamine over the range 32–0·03125 mg/ml were made up daily from a stock solution containing 64 mg/ml of histamine acid phosphate. The first dilution, to 32 mg/ml, was made with a solution of 0·275% sodium bicarbonate. All further dilutions were made with a diluent of 0·275% sodium bicarbonate and 0·8% sodium chloride. The stock solution and both diluents contained 0·4% phenol as a preservative.[15]

HISTAMINE CHALLENGE TESTS

All tests were performed during the afternoon to minimise any effect of circadian rhythms on ventilatory function or bronchial responsiveness. They used a dosimeter technique,[16] each incremental challenge dose comprising five nebulisations (50 μl). Doubling cumulative doses of histamine were administered, beginning with a dose of 1·5625 μg (50 μl of histamine solution, 0·03125 mg/ml).[17] FEV_1 was measured five minutes later and the next histamine dose followed. The challenge sequence was terminated when FEV_1 had fallen by 20% or more or when the maximum cumulative dose (3·2 mg) had been given. No bronchodilator was given at this point.

FEV_1 was remeasured 45 minutes after the completion of test 1. If it had returned to 90% or more of its baseline value, a second baseline was established as the mean FEV_1 from three further sets of measurements at five minute intervals. Test 2 was then carried out in an identical manner to test 1. If FEV_1 had not returned to 90% of baseline, further measurements were taken at five minute intervals and the second histamine test was delayed until this level had been attained.

A "control" test was performed on a separate day at the same time (± 2 hours) as test 1, the two tests being separated by 1–21 days.

EXPRESSION OF BRONCHIAL RESPONSIVENESS

The FEV_1 (ordinate, arithmetic scale) following the inhalation of histamine was plotted against histamine dose (abscissa, logarithmic scale). Bronchial responsiveness, expressed as the cumulative dose of histamine (μg) causing a 20% decrement in FEV_1 (PD_{20}), was calculated from the graph by linear interpolation between the last two values.[18]

STATISTICAL ANALYSIS

For statistical analysis we used the computer program Minitab. Baseline FEV_1 values were compared by Student's t test. A "refractory index" for paired tests was defined by the ratio PD_{20} test 2:PD_{20} test 1, a ratio of 1 indicating no refractoriness. As the distribution of the logarithms of these ratios was skewed, the sign test was used to assess whether the median refractory index differed significantly from 1. The confidence intervals for the median refractory index were calculated from the binomial distribution (p = 1/2; n = 19 or 20). As exact 95% confidence intervals could not be derived from the data, we give the intervals with the lowest level of confidence $\geqslant$ 95%.[19]

Results

After test 1 FEV_1 returned to 90% of its initial baseline within 45–60 minutes in all subjects. The PD_{20} values for each histamine test are shown in the table. In 17 of the 20 subjects PD_{20} test 2 was greater than PD_{20} test 1. Two subjects did not achieve a 20% fall in FEV_1 after the highest dose of histamine during the second test.
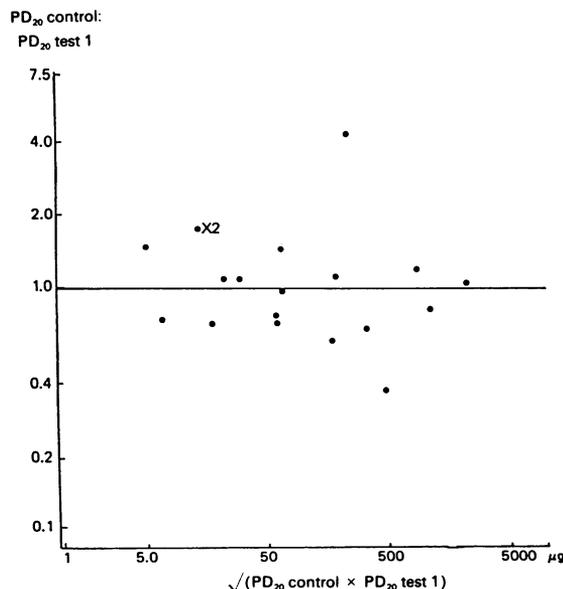

Fig 1 *Relation between the ratio PD_{20} control: PD_{20} test 1 and mean PD_{20} (provocative dose of histamine causing a 20% fall in FEV_1).*

Results of measurements of PD_{20} histamine

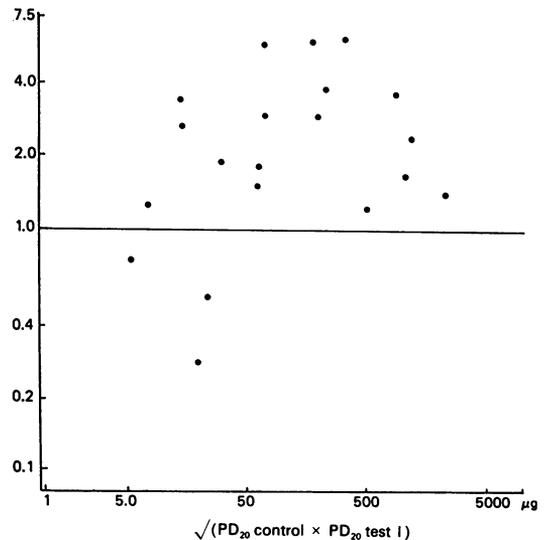| Subject No	Control day (μg)	Test 1 (μg)	Test 2 (μg)	Test 2 with baseline FEV_1 of test 1 (μg)
1	2300	2240	>3200	>3200
2	1050	1300	>3200	2500
3	*	1000	1700	1150
4	950	800	2900	1500
5	460	105	400	280
6	290	800	1000	1000
7	270	400	2440	1900
8	200	180	540	400
9	135	225	1345	1100
10	80	55	165	225
11	65	68	400	300
12	52	68	126	95
13	50	70	110	25
14	30	28	54	30
15	23	21	11	9
16	18	10	27	20
17	18	10	34	65
18	15	21	6	2
19	6	8	10	13
20	6	4	3	3
Geometric means				
Control *v* test 1 (n = 19)	83	82		
Test 1 *v* test 2 (n = 20)		93	189	144

*Subject declined test.

Fig 2   *Relation between the ratio PD_{20} test 2: PD_{20} test 1 (refractory index) and mean PD_{20} (provocative dose of histamine causing a 20% fall in FEV_1).*

These tests were assigned the maximum PD_{20} value of 3200 μg—a procedure that underestimates any refractory effect. The geometric mean PD_{20} for test 2 (189 μg) was greater than that for test 1 (93 μg), and the median refractory index of 2·20 was significantly greater than 1 (96% confidence interval 1·43–3·40; p = 0·003).

The geometric mean control PD_{20} measurements (19 subjects only) was 83 μg, compared with 82 μg for the corresponding test 1 measurements. The PD_{20} control:PD_{20} test 1 ratio (fig 1) was not related to the geometric means of each paired PD_{20} control and PD_{20} test 1 (that is, to the best estimate of the true or "usual" PD_{20}); nor did the median ratio, 1·03, differ significantly from 1 (98% confidence interval 0·71–1·45).

EFFECT OF BASELINE FEV_1 ON DEGREE OF REFRACTORINESS
The mean baseline FEV_1 was significantly lower for test 2 than that for test 1 (3·52 *v* 3·71 litres; p < 0·001). This may have affected the second PD_{20} measurement, as the second challenge was carried out at a time when further recovery in FEV_1 might have been expected after the first test. The dose of histamine represented by PD_{20} test 2 would in these circumstances have been the dose that antagonised any recovery and in addition provoked a 20% decrement in FEV_1. This would increase the value of PD_{20} test 2 and exaggerate the refractoriness measurement.

The potential strength of this possible artefact was examined by recalculating PD_{20} test 2 as a 20% fall from the baseline FEV_1 of test 1 (table). This assumes that during the course of the second test the FEV_1 would have recovered fully to its original baseline had it not been for the second histamine challenge. This produced an amended geometric mean PD_{20} for the second test of 144 μg and an amended median refractory index of 1·75, which still differed significantly from 1 (96% confidence interval, 1·15–2·67; p = 0·012). The median ratio of the original refractory index (2·20) to the amended refractory index (1·75) was also significantly different from 1 (p = 0·013).

RELATION BETWEEN REFRACTORINESS AND PD_{20}
The relation between the refractory index (PD_{20} test 2: PD_{20} test 1) in each subject and the geometric mean PD_{20} values from the control test and test 1 is shown in figure 2. All 14 subjects with a mean PD_{20} above 25 μg showed a refractory index greater than 1. When the amended PD_{20} test 2 values were used, the refractory index consistently exceeded 1 when the mean PD_{20} was above 100 μg.

Discussion

This study has found a diminished bronchoconstrictor response to histamine in most of the 20 asthmatic subjects given a second histamine inhalation test 45–60 minutes after an initial test. We therefore support the

suggestion that histamine provoked broncho-constriction induces refractoriness, at least in some individuals. A refractory index exceeding 1 was not observed uniformly with high levels of bronchial responsiveness (that is, low PD_{20} values), however, and our results suggest a possible PD_{20} threshold of the order of 25–100 μg histamine. Above this threshold the refractory index did not appear to be related to PD_{20}, implying that other factors help to determine the degree of refractoriness, once the PD_{20} (and dose of histamine) are sufficiently high for refractoriness to occur.

The failure of some investigations to show refractoriness is unexplained—unless the subjects in these studies had high levels of airway responsiveness. Adequate numbers of subjects and reasonable precision in the measurement of PD_{20} are necessary if refractoriness is to be recognised, and baseline levels of ventilatory function should be closely similar for the paired tests. Investigations measuring bronchial responsiveness by means other than the $PD_{20}FEV_1$ may not produce as much bronchoconstriction (or give as much histamine), and this too could minimise the refractory effect. Finally, it is not always clear that studies failing to detect a refractory period were carried out on typical asthmatic subjects rather than subjects with relatively fixed airway obstruction attributable to cigarette smoking.[11]

The magnitude of the refractory effect generated by a single histamine test was appreciable though not substantial, the median refractory index being 2·20 (range 0·24–6·1). The range observed above the "threshold" of refractoriness was 1·3–6·1. Refractoriness of this degree could exert an important confounding effect when histamine challenge tests are used repeatedly within a single day to measure bronchial responsiveness. For example, bronchial responsiveness to histamine was shown to increase 1 to 2 hours after exposure to respirable industrial agents associated with occupational asthma, before the onset of the ensuing late asthmatic reactions.[20] Refractoriness here would have diminished the true magnitude of this effect and could have masked it. Investigations showing an apparent circadian rhythm in bronchial responsiveness from frequently repeated histamine tests at uneven intervals may have been subjected to both masking and exaggerating distortions because of the refractory effect. The duration of the refractory period, which Manning *et al* estimated to be of the order of three to six hours, is obviously a critical factor.[9] Until this is established, the importance and strength of the refractory effect as a confounding factor will remain unclear.

It is improbable that our observations could have been generated by an artefact in the histamine delivery system. If the nebuliser is used continuously, its output may be expected to decline owing to cooling, but with intermittent use this is unlikely and we have found no evidence of it in practice. A further possible artefact from repeated nebuliser use is increasing concentration of the histamine solution from evaporation. This is unlikely to be important when solutions are replaced daily, as was the practice in this study. In any event, increasing concentration would tend to mask, not exaggerate, refractoriness.

The failure of some subjects to regain their initial baseline level of FEV_1 before the second histamine test is potentially a more serious flaw. A small degree of residual bronchoconstriction could produce more central deposition of subsequent doses of inhaled histamine, with greater bronchoconstriction because histamine receptors are more concentrated in the central airways.[21] Geometric factors may also have a role, though these effects are more likely to reduce the second PD_{20} measurement.[22] Residual bronchoconstriction provoked by methacholine was found not to influence histamine responsiveness in one study when this was measured on the basis of specific airway conductance.[23]

A major concern is that the FEV_1 may have continued to increase were it not for the second histamine test. This implies that the PD_{20} of the second challenge was amplified by the need to antagonise the continuing recovery and cause a 20% fall in FEV_1 in addition. This would increase the PD_{20} and stimulate or exaggerate a refractory effect. In the present study this effect is unlikely to have exerted a major influence because the FEV_1 had returned to at least 90% of baseline in all patients before the second test was started (mean value 95% of baseline). We did, however, attempt to quantify the size of this effect by recalculating PD_{20} values for the second test using the baseline FEV_1 from the first test. Appreciable refractoriness remained evident even though the decrease in median refractory index from 2·20 to 1·75 was significant. The amended refractory index of 1·75 may represent an overcorrection, however, as it assumes that the baseline FEV_1 would have been regained fully by every subject during the period of the second test and it ignores the other potential confounders that may have exerted a masking influence on refractoriness.

Our findings are supported by observations that both canine[24] and human[25] airway smooth muscle readily develop refractoriness to repeated stimulation with histamine in vitro. Depletion of mediator stores in mast cells, which has been proposed as the mechanism for exercise induced refractoriness,[26] is not likely to be relevant to histamine induced refractoriness. The fact that some degree of refractoriness has been found in response to a wide range of directly and indirectly acting bronchoconstrictors suggests that changes in

the end organ (that is, the airway smooth muscle itself) might be responsible. In the dog this appears to be mediated by a bronchorelaxant prostaglandin, probably PGE_2.[27] The same mechanism may be responsible for histamine tachyphylaxis in human airways,[9] where indomethacin has been shown to increase the steepness of inhaled histamine dose-response curves.[28]

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