

# Hyperventilation-induced asthma: evidence for two mechanisms

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**ABSTRACT** The mechanism by which airway cooling induces airflow obstruction in asthmatic subjects has not yet been established. Using a pair of isocapnic hyperventilation challenges, with a 40-minute interval, we looked for the presence of a refractory period in 19 asthmatic patients (aged 9-18 years). The subjects fell into two groups. The eight in the "non-refractory" group showed less than a 25% reduction in response to the second challenge, but the 11 in the "refractory" group showed at least a 35% reduction. Twelve subjects also performed a hyperventilation challenge after cholinergic blockade with inhaled ipratropium bromide. In five, in whom no refractoriness after hyperventilation was seen, there was a significant protection from cholinergic blockade ( $p < 0.05$ ). In these a vagal (cholinergic) reflex seems likely. The remaining seven, who had a refractory period, received no significant protection from cholinergic blockade and therefore no evidence for the presence of any cholinergic mechanism. We conclude that two mechanisms are responsible for hyperventilation-induced asthma, one of which is a vagal reflex while mediator release may be the other.

The initiating stimulus in exercise-induced asthma is airway cooling brought about by heat and water loss during the increased ventilation of exercise.<sup>1,2</sup> Hyperventilation alone produces airflow obstruction equivalent to that induced by exercise when ventilation and inspired air conditions are the same.<sup>3</sup> The mechanism by which airway cooling leads to bronchoconstriction is, however, uncertain. In many studies anticholinergic drugs have been found to inhibit exercise-induced asthma in about half the asthmatic subjects tested, suggesting that a vagal reflex may play a part.<sup>4-8</sup> As only a proportion of subjects are protected by cholinergic blockade, some additional mechanism, such as mast-cell mediator release, may also be concerned. This idea is supported by the finding that cromoglycate, which stabilises mast cells *in vitro*,<sup>9</sup> has a protective effect in most people with exercise-induced asthma.<sup>5,7,10</sup> Further circumstantial evidence for mediator release in exercise-induced asthma is provided by the refractory period, during which a second exercise test produces less airflow obstruction than the first.<sup>11</sup> It may last up to two hours and one possible explanation is that mast cells require time to regranu-

late.<sup>12</sup> Another is that concentrations of catecholamines rise during exercise and, by increasing cyclic AMP in airways, temporarily protect against exercise-induced asthma.<sup>13</sup> If the latter explanation were true no refractory period would be expected after hyperventilation-induced asthma as no rise in circulating catecholamines has been found with hyperventilation at rest.<sup>14</sup> Possibly both a vagal reflex and mediator release occur after airway cooling, although either may predominate in different patients or even in the same patient at different times.

We looked for the presence of a refractory period after hyperventilation-induced asthma, as evidence of possible mediator release. We have related the presence or absence of refractoriness to the effect of cholinergic blockade on hyperventilation-induced asthma to determine the role of a vagal reflex mechanism.

## Methods

Nineteen patients were selected from the paediatric and adult asthma clinics on the basis of a history of exercise-induced asthma (table). All had multiple positive immediate reactions to skin prick tests for common allergens. Their clinical severity ranged

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Data on the asthmatic patients studied

Patient No	Predicted PEFR (l/min)	Day 1				Day 2			
		HV <sub>1</sub>		HV <sub>2</sub>		HV <sub>C</sub>		HV <sub>1,B</sub>	
		B	R	B	R	B	R	B	R
Refractory group									
1	335	250	95	200	200	215	160	215	165
2	415	340	255	325	300	310	235	330	240
3	420	395	210	325	285				
4	350	265	95	195	130	345	225	400	270
5	450	510	335	425	360				
6	430	330	115	285	200				
7	450	370	200	350	270	370	200	395	240
8	375	365	160	340	245	360	255	370	245
9	450	355	300	360	345	270	230	355	235
10	445	410	335	420	380				
11	370	280	180	255	200	240	135	260	160
Mean	408	352	207	317	265	310	205	332	220
SEM	13.0	22.3	27.0	23.3	23.7	23.5	16.7	26.9	16.2
Non-refractory group									
12	290	285	235	285	245	270	223	315	300
13	330	225	120	250	110				
14	410	280	160	300	150				
15	300	375	290	400	315	360	280	395	380
16	555	455	250	435	250	425	195	540	510
17	415	260	160	245	145	420	335	430	400
18	330	245	125	260	120				
19	340	305	210	310	160	315	230	255	270
Mean	371	304	194	311	162	358	253	387	372
SEM	29.1	25.3	20.6	23.5	28.1	30	24.8	49.0	42.1
Total group									
Mean	393	332	202	315	232	325	225	355	285
SEM	15.1	17.6	17.8	16.6	25.5	19.7	15.1	25.6	29.1

PEFR—peak flow rate; HV<sub>1</sub>, HV<sub>2</sub>—first and second hyperventilation challenge tests with 40-minute interval; HV<sub>C</sub>, HV<sub>1,B</sub>—control hyperventilation challenge test and test repeated after ipratropium bromide; B—baseline PEFR (l/min); R—PEFR at maximum response (l/min).

from those taking occasional  $\beta$ -agonists to those needing an additional prophylactic agent such as cromoglycate, sustained-release aminophylline, or inhaled steroids. None was taking oral steroids. All drugs were discontinued for at least eight hours before the study and sustained-release aminophylline for 24 hours.

The study was approved by the ethics committee of the Hammersmith Hospital and all subjects and their parents gave their informed consent.

The hyperventilation was performed as previously described,<sup>3</sup> the subject voluntarily hyperventilating for five minutes via a dry gas meter (Parkinson Cowan CD4), so that the rotation of the gas meter needle corresponded to the rotation of a superimposed motor-driven pointer, calibrated to give the required minute ventilation ( $\dot{V}$ ). The target  $\dot{V}$  was set at 20 times the FEV<sub>1</sub>, although in some subjects with highly reactive airways a lower target was necessary. The same target was used for each subject with every study. End-tidal carbon dioxide ( $P_{ETCO_2}$ ) was monitored (Capnograph, Godart) throughout the study and 100% carbon dioxide added to the inspired air so that  $P_{ETCO_2}$  was maintained at about 5 kPa (37.5 mm Hg) throughout the

hyperventilation. As the study was performed in an air-conditioned laboratory the inspired air temperature ( $22 \pm 2^\circ\text{C}$ ) and relative humidity ( $50 \pm 5\%$ ) were constant, so airway cooling was proportional to the  $\dot{V}$  achieved. Peak expiratory flow rate (PEFR; Wright peak flow meter, Aired, calibrated against a Rotameter flow meter) was determined in triplicate and the highest values were recorded, before and at the end of hyperventilation, at one, three, and five minutes after finishing, then every five minutes until spontaneous recovery occurred. The response (% fall in PEFR) was taken as the lowest PEFR after the challenge, expressed as a percentage fall from the baseline value:  $((\text{Baseline PEFR} - \text{lowest PEFR})/\text{baseline PEFR}) \times 100\%$ .

Subjects attended on two study days within 14 days. On the first day an initial hyperventilation challenge was performed and this was repeated 40 minutes later with the same target  $\dot{V}$ . When PEFR was back to baseline value a methacholine inhalation challenge was performed by the method that Cockcroft and colleagues have standardised for histamine inhalation.<sup>15</sup> Methacholine, in doubling concentrations, was inhaled until PEFR had fallen by at least 20% from the value after inhalation of saline;

the concentration causing a 20% fall ( $PC_{20}$ ) was calculated. On the second day, an initial control hyperventilation was performed, with the same target  $V$  as on the first day. At least 60 minutes later subjects inhaled ipratropium bromide (80  $\mu$ g, four puffs) from a metered aerosol. Cholinergic blockade was ascertained 45 minutes after the ipratropium bromide by showing that no fall in PEFR was produced by the inhalation of methacholine at a concentration of at least four times the predetermined  $PC_{20}$ . A second hyperventilation challenge, with matched target  $V$ , took place 60 minutes after ipratropium bromide (two hours after the initial hyperventilation challenge to allow time for recovery from refractoriness). The results were analysed with paired and unpaired  $t$  tests and correlations determined by the Rank Spearman correlation coefficient.

## Results

### REPEATED HYPERVENTILATION

There was a significant reduction in the mean response to a second challenge performed 40 minutes after the first ( $p < 0.01$ ), although the  $\dot{V}$  and the baseline PEFR were not significantly different between the two tests (table, fig 1). When the results of individual subjects were analysed, two groups could be differentiated on the basis of the reduction in response to the second challenge. A protection index was calculated that gave the reduction in response to the second hyperventilation challenge ( $HV_2$ ) as a percentage of the initial response ( $HV_1$ ):  $((\% \text{ fall PEFR } HV_1 - \% \text{ fall } HV_2) / \text{fall PEFR } HV_1) \times 100\%$ .

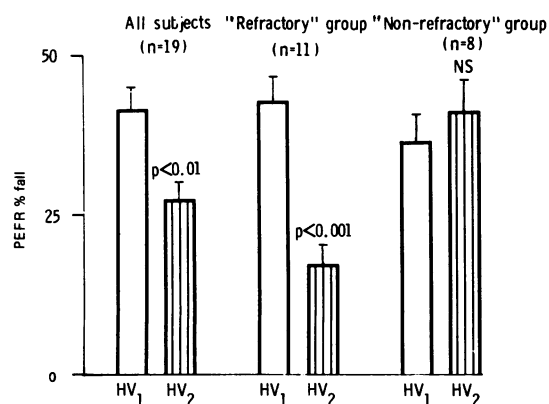


Fig 1 Mean responses to a second hyperventilation challenge ( $HV_2$ ) 40 minutes after the first ( $HV_1$ ) for the whole group and for the two subgroups ('refractory' and 'non-refractory').

Eleven subjects (the 'refractory' group) were defined by a protection of 35% or more after the second challenge. The remaining eight subjects (the 'non-refractory' group) had less than 25% protection. There was no significant difference in the ventilation achieved during the first and the second test in either group: refractory group  $43 \pm 2.2$  (mean  $\pm$  SEM, l/min) and  $41 \pm 2.0$ ; non-refractory group  $41 \pm 1.7$  and  $40 \pm 1.7$  respectively. The baseline PEFR was significantly lower ( $p < 0.02$ ) in the refractory group before the second hyperventilation challenge (table), indicating that these subjects had not fully recovered from the initial challenge by 40 minutes. There was no difference between the two groups in atopic state, clinical severity, baseline PEFR or the magnitude of the bronchoconstrictor response to hyperventilation.

In three patients showing a refractory period a third test 120 minutes after the first showed recovery of the response to hyperventilation.

### EFFECT OF CHOLINERGIC BLOCKADE

In 12 subjects the effect of cholinergic blockade on hyperventilation-induced asthma was investigated. The presence of cholinergic blockade, produced by ipratropium bromide, was confirmed in all these patients by methacholine challenge. The methacholine  $PC_{20}$  of the subjects was  $0.87 \pm 0.27$  mg/ml (mean  $\pm$  SEM). After ipratropium bromide, challenge with methacholine in a mean concentration of  $3.88 \pm 0.83$  mg/ml gave a fall in PEFR of  $1.7 \pm 1.1\%$ , confirming effective cholinergic blockade. The five non-refractory subjects showed a significant reduction in the response to hyperventilation after ipratropium bromide ( $p < 0.05$ ) but in the seven refractory subjects there was no difference between the results of the two tests (table, fig 2). In neither

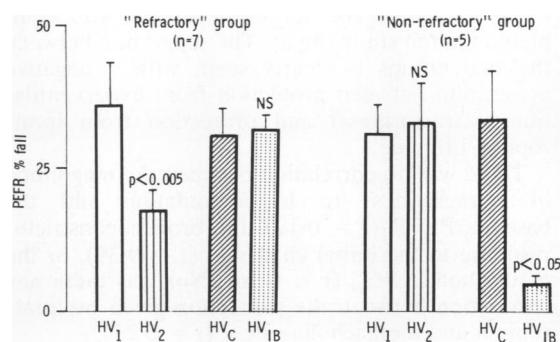


Fig 2 Mean responses to repeated hyperventilation challenge ( $HV_1$  and  $HV_2$ ) in the two subgroups, compared with the protection afforded against hyperventilation challenge by inhalation of ipratropium bromide ( $HV_C$  compared with  $HV_{1B}$ ).

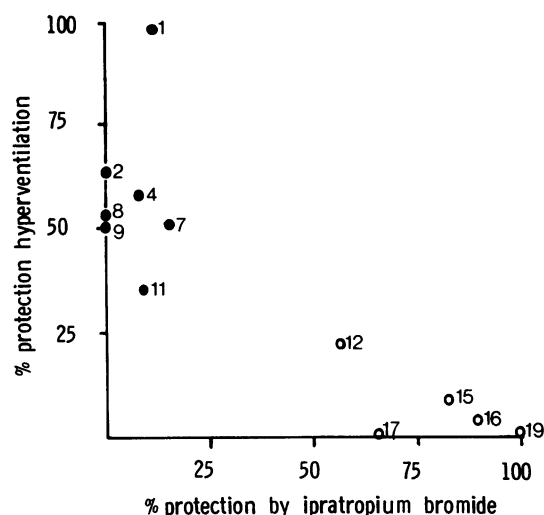


Fig 3 Relation between refractoriness to repeated hyperventilation challenge (% protection hyperventilation) and inhibition of hyperventilation-induced asthma by ipratropium bromide (% protection by ipratropium bromide) for 12 individual subjects. Numbers relate to the table. Closed circles indicate "refractory" subjects and open circles "non-refractory" subjects.

group of subjects was there any significant difference in  $\dot{V}$  achieved between the pair of tests before and after cholinergic blockade: refractory group  $42 \pm 8.2$  (mean value  $\pm$  SEM, l/min) and  $40 \pm 7.4$ ; non-refractory group  $43 \pm 2.9$  and  $45 \pm 3.5$ . The bronchodilator effect of ipratropium bromide was confirmed by the increase in baseline PEFR: refractory group  $14.9 \pm 5.6\%$ ; non-refractory group  $19.0 \pm 4.85\%$ .

The protection index for repeated hyperventilation was plotted against the protection index for cholinergic blockade for each subject who completed the full study (fig 3). The separation between the two groups is clearly seen, with a negative association between protection from hyperventilation (refractoriness) and protection from ipratropium bromide.

There was no correlation between the magnitude of refractoriness to hyperventilation and the baseline PEFR ( $r = 0.12$ ), the bronchoconstrictor response to the initial challenge ( $r = 0.39$ ), or the methacholine  $PC_{20}$  ( $r = 0.16$ ). Nor was there any correlation between the protection given by ipratropium and methacholine  $PC_{20}$  ( $r = 0.27$ ).

#### REPRODUCIBILITY

Nine subjects were tested for the presence of a refractory period after hyperventilation-induced asthma on more than one occasion, from two weeks

up to 12 months after the original test. All five of those in the refractory group were again refractory but only two out of four in the non-refractory group remained non-refractory. One of these was the patient seen in fig 3 to have an intermediate response (subject No 12).

#### Discussion

Using isocapnic hyperventilation we could show in some but not all subjects a diminished bronchoconstrictor response to a second hyperventilation test repeated within 40 minutes. In those who showed no difference in response between the two challenges (the non-refractory group) hyperventilation-induced asthma was significantly inhibited by cholinergic blockade, which suggests that a vagal reflex mechanism was concerned. In those showing a diminished response to the second challenge (the refractory group) there was no protection from cholinergic blockade, which points to another mechanism, such as release of mast-cell mediators. There was no difference in age, atopic state, clinical severity, sensitivity to hyperventilation, or baseline airway function between the two groups, although the refractory group did show a slower recovery from hyperventilation-induced asthma.

Interpretation of comparisons in bronchoconstrictor response when baseline values vary is always difficult. In the repeated hyperventilation challenges the refractory group had a lower baseline PEFR before the second of the pair of tests but it was these subjects who showed a diminished bronchoconstrictor response, making it unlikely that the difference between the two groups could be explained by a difference in baseline values. After inhalation of ipratropium bromide the bronchodilatation was similar in the two groups and could not therefore account for the difference between them in degree of protection. Using the absolute PEFR values instead of percentage changes made no difference to our results (table). Published data that include both PEFR and  $FEV_1$  show that the two measurements give similar results.<sup>8,16</sup>

In previous studies cholinergic antagonists, given in large doses, have been shown to protect (producing at least a 50% reduction in responses) 40–60% of subjects after exercise challenge, although the presence of cholinergic blockade had not been demonstrated by methacholine challenge in many of the studies.<sup>4,8</sup> This study shows protection of a similar degree after hyperventilation challenge. Cholinergic blockade has been proved to be ineffective in protecting against exercise-induced asthma<sup>17</sup> or hyperventilation-induced asthma<sup>18</sup> when subfreezing air is inhaled but there are no



published data on the protective effect of atropine in hyperventilation-induced asthma when ambient air temperatures are used.

Cholinergic blockade was found to inhibit exercise-induced asthma only in those subjects in whom airway obstruction affected mainly large airways, as determined by changes in density dependence of maximum expiratory flow,<sup>6,7</sup> whereas sodium cromoglycate inhibited those with predominantly small airways obstruction.<sup>7</sup> These findings may provide an explanation for our results since the non-refractory group may have large airway obstruction, effected by a vagal reflex, whereas the refractory group may have predominantly small airway obstruction, where mediator release is more likely; and it was this latter group that showed a delayed recovery from hyperventilation-induced asthma.

Evidence that mediator release plays a part in exercise-induced asthma is provided by the demonstration of a rise in plasma histamine concentration<sup>19</sup> and neutrophil chemotactic factor<sup>20</sup> during and after exercise in asthmatic but not normal subjects. No rise in either plasma histamine<sup>19</sup> or neutrophil chemotactic factor<sup>21</sup> has been found in hyperventilation-induced asthma, however, which suggests that the rise in plasma concentrations during exercise may reflect release from basophils rather than from pulmonary mast cells. The failure of venous plasma concentrations of mediators to rise during hyperventilation does not exclude undetected local pulmonary mediator release. Mediators may have a major role in the production of exercise-induced asthma in only a proportion of subjects as not all are protected by cromoglycate<sup>10</sup> and the inhaled antihistamine clemastine gave a 50% protection from exercise-induced asthma in only five out of 10 subjects tested.<sup>16</sup> The reason for the refractory period in exercise-induced asthma is uncertain, but one explanation is that mediators could be released during the first exercise test and time is required for mast cells to regranulate or to recover their response.<sup>12</sup>

In a study by Weiller-Ravell and Godfrey only half the subjects made refractory to exercise by multiple testing responded to a concentration of inhaled allergen that had previously caused airflow obstruction.<sup>22</sup> This not only is further evidence of a dual mechanism in exercise-induced asthma but suggests that in some subjects exercise-induced asthma and the response to inhaled allergens share a common pathway. It is unlikely that airway muscle becomes refractory to repeated challenge, since the response to histamine challenge during the refractory period is unchanged.<sup>23</sup>

There is much evidence to suggest that exercise-

induced asthma and hyperventilation-induced asthma have the same pathogenesis.<sup>2,3,13</sup> After hyperventilation-induced asthma, however, Stearns *et al* found no evidence for refractoriness in seven adults with mild asthma, although a refractory period after exercise-induced asthma was observed in the same subjects.<sup>13</sup> They concluded that mediators were not concerned in the pathogenesis of exercise-induced asthma or hyperventilation-induced asthma and that the refractory period after exercise was caused by the rise in circulating endogenous catecholamines, which activate airway  $\beta$ -receptors. This explanation is doubtful, since it has been shown that exercise in warm, humid air to prevent bronchoconstriction, presumably giving the same catecholamine response, is not followed by refractoriness.<sup>24</sup> Furthermore, the plasma catecholamine and cyclic AMP response of asthmatics to exercise appears to be blunted, and as no rise in plasma catecholamines occurs with hyperventilation this is an unlikely explanation.<sup>14</sup> Our finding of a refractory period after hyperventilation is at variance with the results of Stearns *et al*<sup>13</sup> and may reflect a greater severity of asthma in our subjects. If mediator release plays a part in the airflow obstruction resulting from airway cooling in at least some subjects, it would explain why these subjects are not protected by cholinergic blockade. Cromoglycate may have prevented hyperventilation-induced asthma in this group, but it was not used as there is some doubt whether it acts solely as a mast cell stabiliser and some evidence for its effect on post-ganglionic cholinergic fibres.<sup>25</sup>

Although the reason for the refractory period remains in doubt, we could divide our subjects into two groups on the basis of their degree of refractoriness to repeated hyperventilation and their protection or lack of protection by cholinergic blockade. This is strong evidence that two mechanisms play a part in hyperventilation-induced asthma even though they may be interrelated (cholinergic stimulation increasing mediator release<sup>26</sup> and histamine being known to stimulate cholinergic reflexes in vagal afferent pathways<sup>27</sup>).

We showed that seven out of nine subjects showed a reproducible degree of refractoriness to repeated hyperventilation on repeated testing; but further studies are needed to look at the short-term and long-term reproducibility and the factors affecting it, as well as the reproducibility of protection by cholinergic agents in hyperventilation-induced asthma. This dual response to airway cooling should, however, be borne in mind in both the treatment and the assessment of agents designed to protect against exercise-induced or hyperventilation-induced asthma.

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