Increased responsiveness to methacholine and histamine after challenge with ultrasonically nebulised water in asthmatic subjects

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ABSTRACT Responsiveness to inhaled methacholine was compared before and 40–60 minutes after a challenge with ultrasonically nebulised water (UNH_2O) in 16 asthmatic patients. The sensitivity to methacholine increased after UNH_2O challenge ($p < 0.001$). The mean dose of methacholine producing a 20% fall in forced expiratory volume in one second was 0.4 (95% confidence limits 0.2, 0.8) μmol, compared with 0.9 (95% confidence limits 0.5, 1.6) μmol in the first methacholine challenge. When the study was repeated in six asthmatic patients with histamine substituted for methacholine, five of the patients were significantly more sensitive to histamine after UNH_2O challenge. It is concluded that challenge with UNH_2O produces an increase in airway responsiveness.

Asthmatic subjects develop bronchoconstriction in response to ultrasonically nebulised hypo-osmolar and hyperosmolar solutions.[1][2] Anderson *et al*[2] reported that the inhalation of up to 24 ml of ultrasonically nebulised water (UNH_2O) induced a reduction in the forced expiratory volume in one second (FEV_1) greater than 20% of the prechallenge level in 70 patients with asthma and that 56 ml of water failed to produce any such reaction in non-asthmatic individuals.[3] Although the mechanism by which UNH_2O produces bronchoconstriction is unknown, the airway response in asthmatics to both exercise and UNH_2O has been shown to be associated with a rise in neutrophil chemotactic activity in serum.[4][5]

In a series of experiments in rabbits, Irvin *et al*[6] investigated the effects of the inflammatory response on histamine induced increases in airway resistance. Cumulative dose-response curves for histamine were obtained in anaesthetised animals before and after the administration of C5a desarg, a serum derived chemoattractant for neutrophils. The sensitivity to histamine increased after C5a desarg administration. When histological sections of these rabbit airways were examined, there was a florid accumulation of neutrophils both in airway epithelium and in and around the smooth muscle.

We reasoned that if UNH_2O challenge is associated with an increase in neutrophil chemotaxis bronchoactive substances may be produced by the attracted neutrophils, leading to a change in sensitivity of the bronchial smooth muscle. If this were so, UNH_2O challenge might be expected to potentiate the response to bronchoconstricting agonists such as methacholine and histamine. In addition, if UNH_2O challenge is associated with an acute inflammatory response, this in turn could alter epithelial permeability, allowing greater access of methacholine and histamine to smoth muscle receptor sites. This study investigates the effect of a prior challenge with UNH_2O on the bronchoconstrictor response to methacholine and histamine in asthmatic patients.

Methods

We studied 22 asthmatic patients whose reversible airways obstruction had been confirmed during a visit to the respiratory laboratory at Royal Prince Alfred Hospital and who agreed to return for subsequent visits. The study protocol was approved by the ethics review committee, and all patients agreed to withhold medications for four to six hours before the challenge procedures. Details of the patients and

Table 1 *Details of patients, their maintenance treatment, and predicted normal values for FEV$_1$*

Patient No	Sex	Age	Treatment	Pred FEV$_1$
1	M	29	S, B	3.69
2	F	22	P, S, B, I, T	3.03
3	F	23	S, B	3.34
4	M	36	F, B, C	3.52
5	M	43	S, T	3.13
6	F	36	S, B	2.27
7	F	22	S	2.73
8	F	23	—	3.05
9	F	38	S, B	2.40
10	F	22	S, B	2.89
11	M	22	S, B	4.91
12	M	39	S, T	3.50
13	F	42	S	2.73
14	M	60	S, T	3.21
15	M	25	S, T	4.54
16	M	47	S, B	3.54
17	M	52	S, B	3.40
18	M	33	S, B	3.69
19	F	36	S, B	3.00
20	F	48	S	2.47
21	M	30	S	4.57
22	M	23	S, B	4.48

S—salbutamol; B—beclomethasone dipropionate; P—prednisone; I—ipratropium bromide; T—theophylline; F—fenoterol; C—sodium cromoglycate.

their maintenance treatment are shown in table 1.

Each subject visited the laboratory on two occasions within a period of two weeks, when possible at the same time of day. On one visit a methacholine inhalation test was performed (M$_1$) and on the other occasion a methacholine inhalation test (M$_2$) followed a challenge with UNH$_2$O. This protocol was followed in patients 1–16. In six patients (17–22) histamine was substituted for methacholine.

Resting lung function and changes induced by the challenge procedures were monitored with a Cavitron spirometer (California USA), which measured forced expiratory volume in one second (FEV$_1$) and forced vital capacity (FVC). At the start of the methacholine or histamine inhalation challenge resting FEV$_1$ and FVC were measured. Several forced expirations were performed, until reproducible values were obtained. Values for resting lung function were then expressed as a percentage of predicted values according to Morris *et al*.[7]

Two inhalations of a 0.9% w/v saline solution at room temperature were administered via a de Vilbiss No 40 hand held nebuliser. Patients inhaled from functional residual capacity towards total lung capacity, during which time the bulb of the nebuliser was squeezed by the operator. The patient held the breath for two to three seconds and then exhaled slowly. FEV$_1$ was measured one minute later. The challenges were conducted in a manner similar to that described by Yan *et al*.[8] Histamine and methacholine solutions of 0.625%, 2.5% and 5% w/v were prepared. Patients received initially one

and then three inhalations of the 0.625% solution, three inhalations of the 2.5%, and three inhalations of the 5% solution. When one dose consisted of more than one inhalation these were administered in consecutive breaths. The FEV$_1$ was measured one minute after each dose, and the highest of two or three measurements recorded. On the basis of previous reports on the output of the nebuliser and the concentration of the solution[8] the delivered cumulative doses of methacholine were calculated to be 0.096, 0.385, 1.54, and 6.12 μmol for methacholine and 0.06, 0.24, 0.98, and 3.9 μmol for histamine. In one patient (No 16) the cumulative methacholine dose was increased to 13 μmol. The challenge was stopped when the FEV$_1$ had fallen by more than 20% of the prechallenge, postsaline level. The dose of methacholine or histamine required to induce a 20% reduction in FEV$_1$ (PD$_{20}$) was determined by extrapolation from a curve constructed to relate change in FEV$_1$ to the cumulative dose of methacholine or histamine inhaled.

The UNH$_2$O challenge was carried out with the MistO$_2$gen ultrasonic nebuliser EN 143A (California, USA), which delivered about 1 ml of aerosolised water for each 10 l of air inhaled. The technique used for UNH$_2$O challenge has been described in detail.[2] Before inhaling UNH$_2$O and after measurement of resting FEV$_1$ and FVC as above, the patient inhaled 40 l of room air. No subject had a fall in FEV$_1$ of 15% or greater from initial values after this preliminary test.

Initially the patient inhaled 0.5 ml of aerosolised water. Thirty seconds later the FEV$_1$ was measured and the highest of two or three estimations was recorded. If the fall in FEV$_1$ was less than 10% the patient received doses of about 1, 2, 4, 8, 8, and 8 ml of water until the FEV$_1$ had fallen by 20% or a total of 31 ml had been inhaled. If at any point the FEV$_1$ fell by 10% the challenge proceeded more slowly—that is, the increments were halved. The reduction in FEV$_1$ was expressed as a percentage of the prechallenge post-room air value.

After the UNH$_2$O challenge the patients were allowed to rest for 40–60 minutes. At this time a second methacholine or histamine challenge test was performed and again the PD$_{20}$ was estimated from the relationship between percentage fall in FEV$_1$ and dose of methacholine or histamine. The log of this PD$_{20}$ was compared with the log of the PD$_{20}$ from the first methacholine or histamine challenge by using Student's *t* test for correlated data. Analysis of variance was used to compare FEV$_1$ values before the three challenge tests. The significance of correlations was examined by means of least squares regression analysis. Results were considered significant when $p < 0.05$.
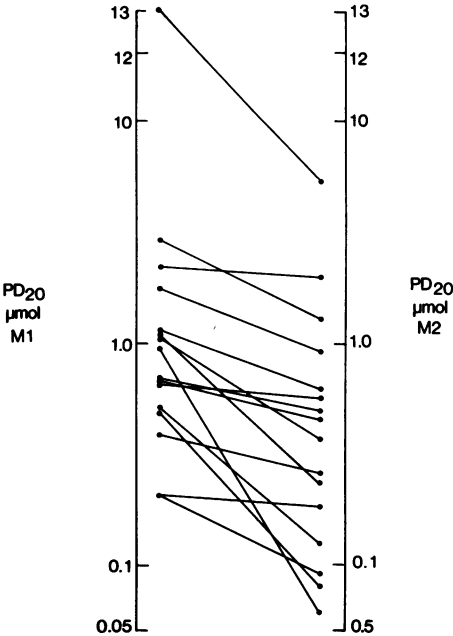
Fig 1   *Dose of methacholine (PD_{20}) required to produce a 20% fall in FEV_1 in the two methacholine challenges (M_1 and M_2) in 16 patients.*

Results

All patients showed a fall in FEV_1 of more than 20% with both M_1 and M_2. The FEV_1 fell after UNH_2O challenge by 18–40% (mean (SD) 30.5% (6.7%)). There was no significant difference for the group in the prechallenge FEV_1 values expressed as a percentage of the predicted values before the two
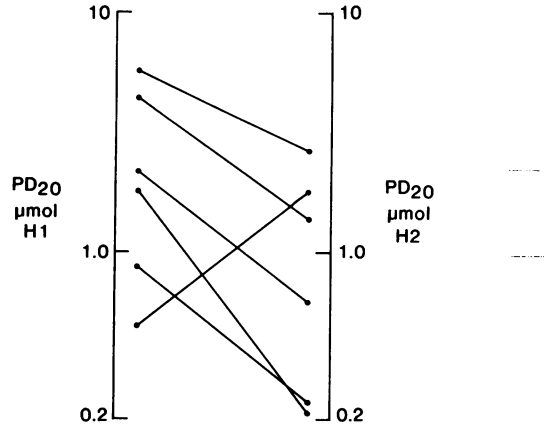

Fig 2   *Dose of histamine (PD_{20}) required to produce a 20% fall in FEV_1 in the two histamine challenges (H_1 and H_2) in six patients.*

methacholine tests (M_1 85% (16%) and M_2 80.8% (16.3%)) and before the UNH_2O challenge (90% (13%)); but the latter value was significantly higher than that before M_2 ($p < 0.001$). In all 16 patients the PD_{20} for M_2 was lower than that for M_1 (fig 1). The mean PD_{20} (95% confidence limits) for the group was 0.9 (0.5, 1.6) μmol for M_1 and 0.4 (0.2, 0.8) μmol for M_2; these values are significantly different ($p < 0.001$). Values for individual patients are shown in table 2.

There was no significant correlation between the resting FEV_1 expressed as a percentage of predicted values and the PD_{20} for either M_1 ($r = 0.09$, $p > 0.05$) or M_2 ($r = 0.19$, $p > 0.05$) or between actual FEV_1 values and the PD_{20}. There was, how-

Table 2   *FEV_1 expressed as a percentage of predicted normal value (% pred) before the first methacholine challenge (M_1), before the second methacholine challenge (M_2), and before the water challenge (H_2O) and dose of provoking agent producing a 20% fall in FEV_1 (PD_{20}—shown in μmol for M_1 and M_2 and in ml for H_2O)*

Patient No	FEV_1 (% pred)			% fall in FEV_1			PD_{20}		
	M_1	H_2O	M_2	M_1	H_2O	M_2	M_1	H_2O	M_2
1	81.6	89.7	69.1	42.5	34.4	37.3	0.40	1.47	0.27
2	88.4	84.8	71.6	26.5	36.6	41.5	0.52	0.69	0.13
3	88.3	109.8	88.0	26.4	29.1	39.1	0.65	14.56	0.61
4	76.1	77.6	67.3	28.3	25.6	40.5	1.05	4.09	0.40
5	75.7	85.9	79.2	61.6	36.8	55.6	0.21	0.51	0.20
6	80.3	88.3	65.4	61.9	40.7	52.3	0.68	1.20	0.48
7	101.5	107.3	102.9	41.1	38.6	43.1	0.22	0.75	0.09
8	120.6	117.7	111.5	30.4	29.1	34.4	1.10	7.47	0.25
9	63.3	73.8	60.8	44.7	34.4	47.3	0.50	<0.95	0.08
10	104.8	84.1	72.6	32.7	34.5	27.1	0.98	<0.75	0.06
11	51.5	65.3	64.4	26.0	23.0	34.0	0.70	3.70	0.52
12	89.0	112.6	109.7	21.0	20.9	25.0	2.90	9.00	1.30
13	98.9	102.6	95.0	28.0	26.0	44.0	2.18	4.00	2.05
14	74.5	86.9	73.8	27.2	26.6	30.0	1.75	4.20	0.94
15	89.2	82.6	84.4	24.7	19.0	23.5	1.15	3.30	0.64
16	82.0	81.0	77.0	22.0	18.0	21.0	13.50	31.00	5.40

Table 3 *FEV$_1$ expressed as a percentage of predicted normal value (% pred) before the first histamine challenge (H$_1$), before the second histamine challenge (H$_2$), and before the water challenge (H$_2$O) and dose of provoking agent producing a 20% fall in FEV$_1$ (PD$_{20}$—shown in μmol for H$_1$ and H$_2$ and in ml for H$_2$O)*

Patient No	FEV$_1$ (% pred)			% fall in FEV$_1$			PD$_{20}$		
	H$_1$	H$_2$O	H$_2$	H$_1$	H$_2$O	H$_2$	H$_1$	H$_2$O	H$_2$
17	70.0	66.8	61.0	20.0	21.0	41.0	0.50	0.95	1.80
18	99.0	93.2	88.0	31.0	14.2	26.0	4.50	>31.50	1.40
19	77.0	85.0	83.7	20.0	22.0	22.0	1.80	1.40	0.22
20	102.0	97.6	95.0	26.0	24.0	20.0	0.88	1.20	0.24
21	99.0	94.5	85.0	29.0	16.0	28.0	5.80	>31.50	2.75
22	86.0	76.8	75.2	42.0	36.0	39.8	2.20	10.60	0.63

ever, a significant correlation between the PD$_{20}$ for M$_1$ and that for M$_2$ (r = 0.8, p < 0.001). The percentage fall in FEV$_1$ in response to UNH$_2$O correlated significantly with the PD$_{20}$ for M$_2$ (r = 0.8, p < 0.001) and the PD$_{20}$ for M$_1$ (r = 0.8, p < 0.001). There was no significant correlation (r = 0.2, p > 0.05) between the percentage fall in response to UNH$_2$O and the increased sensitivity to methacholine, nor was there a correlation (r = 0.4, p > 0.05) between the percentage difference in FEV$_1$ before the two methacholine challenges and the percentage difference in the PD$_{20}$ values.

Of the six patients in whom sensitivity to histamine was studied, five were more sensitive to the histamine challenge performed after UNH$_2$O challenge (H$_2$) than to the first histamine challenge (H$_1$) (fig 2). The PD$_{20}$ to histamine in the remaining patient increased after UNH$_2$O challenge from 0.5 μmol to 1.8 μmol. When the results for the six patients were analysed, there was no significant difference (p > 0.05) between the mean PD$_{20}$ values for H$_1$ and H$_2$. Individual values for the histamine study are shown in table 3. All patients showed a fall in FEV$_1$ in response to UNH$_2$O challenge, ranging from 14% to 36% of the prechallenge level. All patients recovered to within 10% of prechallenge levels of FEV$_1$ after UNH$_2$O and before the commencement of H$_2$. There was no significant difference between resting FEV$_1$ before the two histamine challenges (p > 0.05).

Discussion

This study has shown that patients with asthma have increased sensitivity to inhaled methacholine when it is administered after a challenge with UNH$_2$O. Moreover, this finding was not specific for methacholine as a similar result was obtained in a small group of patients in whom histamine was the provoking agonist. In some patients the decrease in PD$_{20}$ with the second methacholine challenge was small, but the change in the PD$_{20}$ was always in the same direction. There is no apparent explanation for

the finding that patient 17 did not show an increased sensitivity to histamine.

The increased sensitivity to these agonists was not merely a reflection of increased airway tone after the UNH$_2$O challenge.[9][10] Although the mean FEV$_1$ before the second methacholine challenge was significantly lower than that before the UNH$_2$O challenge, there was no difference between the mean values for the starting airway calibre before the two methacholine challenges. In some patients the FEV$_1$ was lower before M$_2$, but this was not a consistent finding. All patients, however, showed an increased sensitivity to methacholine after UNH$_2$O challenge. Moreover, there was no correlation between the baseline lung function and the response to methacholine as determined by the PD$_{20}$, or between the percentage difference in the FEV$_1$ before the two methacholine challenges and the percentage difference in PD$_{20}$. Although the percentage fall in FEV$_1$ after UNH$_2$O correlated significantly with the PD$_{20}$ for M$_2$ a correlation was also evident for M$_1$, implying that it related to the sensitivity to challenge rather than to a change in responsiveness. An increase in responsiveness is not universal with a second challenge—in fact, an appreciable decrease in responsiveness to repeated challenge with UNH$_2$O performed 40–60 minutes after the first challenge has been recorded.[2] Hahn *et al*[11] found that prior exercise challenge did not affect subsequent histamine reactivity, and others have reported[12] that methacholine reactivity was unaltered during the refractory period after exercise or hyperventilation induced asthma.

Our findings could result from inherent variability in the methacholine and histamine inhalation tests. Yan *et al*[8] have found that when histamine inhalation tests are carried out on two separate days, the PD$_{20}$ FEV$_1$ values are highly reproducible when a de Vilbiss nebuliser is used. There is no reason to suspect that findings with methacholine would be different, especially as asthmatic subjects show similar responsiveness to these two agonists.[13] Again, the fact that an increase in responsiveness was found in

21 of 22 patients would seem to diminish the importance of variability in our findings.

Differences in the time elapsed since the last bronchodilator treatment are unlikely to explain our results. This was kept constant for the two challenges. Although the four to six hour time interval may not have eliminated the influence of theophylline, this factor would have operated equally during M_1 and M_2 in these five patients.

The mechanism by which UNH_2O challenge itself induces bronchoconstriction is not known. The fact that aerosols of hypertonic saline also induce asthma favours a change in osmotic environment of the airways as being important in the chain of events that leads to contraction of airway smooth muscle. There are several observations supporting the idea that mediators derived from mast cells play a part and that time is taken to replenish these. Thus the response to UNH_2O is appreciably inhibited by sodium cromoglycate and patients have significantly less response to the same dose of UNH_2O 40 minutes after challenge.[2] We have documented a considerable change in neutrophil chemotactic activity in asthmatics but not normal subjects[5] in response to a challenge with UNH_2O. The potentiating effect of a water challenge on methacholine and histamine responsiveness may relate to the inflammatory changes brought about by the release of histamine and other substances from mucosal cells sensitive to changes in the osmotic environment. Mast cells release mediators in response to changes in osmolarity, although they are thought to be more sensitive to hyperosmolar than hypoosmolar challenges.[14 – 17]

An inflammatory response to the initial stimulus could account for the increase in non-specific bronchial responsiveness in this study. Holtzman *et al*[18] have shown an association between inflammation and hyperresponsiveness in experiments carried out in dogs. Methacholine challenge tests were performed in dogs before and after exposure to ozone. In dogs showing an increased responsiveness to methacholine subsequent histological examination of the airways revealed an inflammatory response with recruitment of neutrophils. Moreover, those dogs that were not hyperresponsive showed no evidence of airway inflammation. The increase in methacholine responsiveness was apparent only one hour after ozone challenge, an interval similar to that in our study. Furthermore, activated complement fragments such as C5a desarg have been shown to produce airway hyperresponsiveness in rabbits,[6] again suggesting an association between airway inflammation and increased airway muscle responsiveness.

In the present study we were not able to investi-

gate the duration of the increased airway responsiveness after UNH_2O challenge. The studies with ozone in dogs[18] have shown that the increased responsiveness to methacholine detectable one hour after ozone exposure is absent one week later. Further studies are necessary to determine whether the alteration in airway responsiveness induced by UNH_2O challenge persists for longer than one to two hours.

The mechanism by which the inflammatory process augments airway reactivity is not apparent. It could arise from reflex stimuli resulting from a lowered threshold in nerve endings. It is possible that inflammatory mediators released from mast cells, from neutrophils themselves, or from airway epithelial cells "prime" the smooth muscle. Neutrophils are known to produce leukotrienes and these in turn are known to augment responses of airway smooth muscle to other agonists.[19] Possibly the increase in airway responsiveness observed in the present study is the result of changes in epithelial permeability. Mediators released in response to UNH_2O challenge may have altered airway epithelial permeability, thus allowing greater access to the histamine and cholinergic receptor sites of the smooth muscle. Exposure to cigarette smoke alters epithelial permeability and has been shown to result in increased responsiveness to histamine in guinea pigs.[20] Demonstration of this, however, requires the presence of beta-adrenergic and parasympathetic antagonists. Others have shown that subjects who smoke exhibit increased permeability but not increased reactivity.[21]

Borland *et al*[22] found a significant reduction in the clearance time for technetrium 99m labelled DTPA after challenge with UNH_2O but not with saline or cold air, which suggests that UNH_2O results in an increase in permeability. Others have reported that osmotic gradients can lead to disruption and swelling of epithelial tight junctions.[23 – 25]

In conclusion, our study has shown that responsiveness to methacholine was increased after UNH_2O challenge. This was not specific for methacholine as results were similar when histamine was the provoking agent. Further studies are necessary to determine the mechanism underlying these findings.

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