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Relation of the hypertonic saline responsiveness of the airways to exercise induced asthma symptom severity and to histamine or methacholine reactivity

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Abstract

Background **Conflicting views exist over whether responsiveness of the airways to hypertonic saline relates to non-specific bronchial hyperresponsiveness measured by histamine or methacholine challenge. The bronchoconstrictor responses to exercise and hypertonic saline are reported to be closely related, but the relationship between the symptoms of exercise induced asthma and airway responsiveness to hypertonic saline is not known.**

Methods **In 29 asthmatic patients with a history of exercise induced asthma, the response to an ultrasonically nebulised hypertonic saline (3·6% sodium chloride) aerosol, measured as the volume of hypertonic saline laden air required to produce a fall in forced expiratory volume in one second (FEV_1) of $\geqslant$20% (PD_{20}), was compared with the concentration of histamine (PC_{20}; group 1) and methacholine (PC_{20}; group 2) producing a 20% fall in baseline FEV_1 and exercise induced asthma symptom severity score (groups 1 and 2). The hypertonic responsiveness was determined in a dose-response manner to a maximum dose of 310 l and the exercise induced asthma symptom severity was scored on a scale of 0–5.**

Results **Of the 29 patients, 23 (79%) were responsive to the hypertonic saline, with PD_{20} values ranging from 9 to 310 l. A significant correlation was found between the PD_{20} hypertonic saline and the exercise induced asthma symptom score. There was no significant correlation between the PD_{20} response to hypertonic saline and the histamine PC_{20} or methacholine PC_{20}. The exclusion of those subjects who failed to respond to hypertonic saline improved the relationship between hypertonic saline and methacholine PC_{20}. No significant correlation was found between the exercise induced asthma symptom score and histamine PC_{20} or methacholine PC_{20}.**

Conclusion **These findings suggest that hypertonic saline responsiveness bears a closer relationship to the severity of exercise induced asthma symptoms than to the non-specific bronchial hyperresponsiveness measured by histamine or methacholine reactivity.**

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Exercise frequently induces bronchoconstriction in asthmatic subjects. It has been suggested that exercise induced asthma results from a transient increase in the osmolarity of the airway periciliary lining fluid caused by the high rate of water loss from airways during conditioning of inspired air to body temperature and full humidity.[1] A number of studies have shown that challenge of asthmatic airways with hypertonic saline aerosol produces a reduction of 20% or more in the forced expiratory volume in one second (FEV_1) in 70–100% of asthmatic subjects, confirming that hyperosmolarity is a potent stimulus for bronchoconstriction.[2–6] The bronchoconstrictor response to exercise and hypertonic aerosol are reported to be closely related.[6] At least one component thought to contribute to the magnitude of the exercise response in asthma is the underlying level of non-specific bronchial hyperresponsiveness. While some studies have shown a correlation between the two responses,[7 8] others have not.[6 9] Conflicting views also exist over whether responsiveness of the airways to hypertonic saline relates to bronchial hyperresponsiveness measured by histamine or methacholine challenge. Smith *et al*[5] showed a significant correlation between the methacholine reactivity and the hypertonic response in a group of asthmatic subjects with moderate to severe bronchial hyperresponsiveness (mean PD_{20} methacholine, 0·68 μmol). Belcher *et al*[6] have reported similar results for histamine. Using a quantitative method to determine the hypertonic saline response in asthmatic subjects and a wider range of methacholine reactivity, Boulet *et al*, however, could not establish any correlation between the hypertonic saline response and methacholine reactivity.[4]

In this study we have assessed the contribution of non-specific bronchial hyperresponsiveness to the hypertonic saline response in asthmatics by re-examining the relationship between the hypertonic saline response and histamine and methacholine reactivity. We have also studied the relationship between the perceived severity of symptoms of exercise induced asthma and airway responsiveness to hypertonic saline, histamine, and methacholine.

Methods

PATIENTS

Twenty nine asthmatic patients (17 male, 12 female) of mean (SE) age 29·6(1·7) years,

with a clear history of exercise induced asthma, participated in the study. All were asked to score the severity of their symptoms of exercise induced asthma over the previous three months on a scale of 0–5 based on asthma severity scores used in previous studies[10–12] (0=no symptoms, 1=mild, 2=mild to moderate, 3=moderate, 4=moderate to severe, 5=severe). They were all non-smokers and 28 were atopic on skin prick testing as judged by >3 mm weals in response to at least one of five common allergens (mixed grass pollen; *Dermatophagoides pteronyssinus*; dog, feather, and cat extracts: Bencard, Brentford, Middlesex, UK). Fifteen patients were taking inhaled β_2 agonists alone as required, while 14 also used regular inhaled corticosteroid (beclomethasone dipropionate). Their mean (SE) percentage predicted FEV_1 was 92·9(2·4). They were randomly allocated to one of two groups to be challenged with either histamine (group 1) or methacholine (group 2).

Group 1 consisted of 15 patients, 11 male and four female, of mean (SE) age 29·7 (3·9) years with a mean (SE) percentage predicted FEV_1 of 92·3 (3·1) (table 1).

Group 2 consisted of 14 patients, six male and eight female, of mean (SE) age 29·6 (2·6) years with a mean (SE) percentage predicted FEV_1 of 93·6 (3·9) (table 2).

All the subjects gave written informed consent and the study was approved by Southampton University and Hospitals Joint Ethical Subcommittee.

STUDY DESIGN

On the first visit the patients in group 1 received a histamine challenge while those in group 2 received a methacholine challenge. Within one week of their first visit, all returned to the laboratory at the same time of day as on their first visit and received a hypertonic saline challenge. Inhaled β_2 agonists were withheld for eight hours before each visit to the laboratory; topical corticosteroids were continued as usual.

Bronchial provocation

Histamine/methacholine challenge This was performed with the five breath technique modified from that of Chai and colleagues.[13] The lowest concentration of histamine monophosphate or methacholine used was 0·03 mg/ml, and doubling concentrations were administered to a maximum of 16 mg/ml. Before the challenge a baseline FEV_1 was obtained, which was the highest of three technically satisfactory recordings obtained with a dry wedge spirometer (Vitalograph, Buckingham, UK). The patients were then instructed to take five deep breaths of aerosol from functional residual volume to total lung capacity from an Inspiron nebuliser, from which normal saline was nebulised with compressed air at a flow rate of 8 l/min. Two measurements of FEV_1 were performed at three minutes, with the higher value being accepted. If the FEV_1 obtained after saline administration was within 10% of baseline FEV_1, the histamine or methacholine challenge was undertaken. Increasing doubling concentrations of agonist were administered at three minute intervals until a 20% fall in FEV_1 from the value obtained after saline administration was recorded. From a plot of the percentage fall in FEV_1 against the natural logarithm of the cumulative agonist concentration, the provocation concentration of histamine or methacholine giving a 20% fall in FEV_1 (PC_{20}) was derived by linear interpolation.

Hypertonic saline challenge The nebuliser used to deliver hypertonic saline was a DeVilbiss model 65 ultrasonic nebuliser (DeVilbiss, Feltham, Middlesex, UK). We have previously shown that the output of hypertonic saline to the mouthpiece is linearly related to the volume of air drawn through the nebuliser, with an output of 10·8 ml/100 l of air at the chosen nebuliser setting.[14]

The hypertonic saline (3·6% NaCl) challenge was given in a dose-response manner. Two measurements of FEV_1 were made for each patient, and the higher reading was used

Table 1 Baseline percentage of predicted FEV_1, exercise induced asthma (EIA) symptom score and airway response to histamine and hypertonic challenge (group 1)

Patients No.	*Age (years)*	*Sex*	*FEV_1 (% predicted)*	*EIA symptom score (0–5)*	*PC_{20}(H) (mg/ml)*	*PD_{20} (3·6% NaCl) (litres)*
1	24	M	77	3	2·80	155
2	34	F	108	3	3·40	36
3	30	M	100	4	0·22	9
4	18	M	77	4	0·68	101
5	36	M	99	2	2·41	231
6	43	M	92	4	0·89	9
7	23	F	105	5	13·7	139
8	36	M	85	4	1·99	26
9	23	M	73	3	0·19	124
10	33	M	78	5	0·25	157
11	22	F	109	4	2·70	10
12	25	F	101	4	2·60	59
13	54	M	97	3	1·28	295
14	33	M	98	2	1·70	NR
15	21	M	85	3	0·41	NR
Mean	29·66		92·27		*1·16	
SEM	3·87		3·14			

*Geometric mean.
PC_{20}(H)—concentration of histamine producing a 20% fall in the baseline FEV_1; PD_{20}(3·6% NaCl)—volume of hypertonic saline laden air required to produce 20% fall in the baseline FEV_1; NR—non-responsive; FEV_1—forced expiratory volume in one second; EIA—exercise induced asthma.

Table 2 Baseline percentage of predicted FEV_1, exercise induced asthma (EIA) symptom score and airway response to methacholine and hypertonic challenge (group 2)

Patient No	Age (years)	Sex	FEV_1 (% predicted)	EIA symptom score(0–5)	$PC_{20}(M)$ (mg/ml)	PD_{20} (3·6% NaCl) (litres)
16	31	F	77	4	0·06	25
17	25	F	97	5	0·17	88
18	24	F	84	5	5·78	102
19	18	M	95	5	0·25	72
20	25	M	125	4	0·92	310
21	23	F	89	4	0·34	83
22	24	F	76	3	0·04	60
23	39	M	106	4	1·43	33
24	30	F	101	2	4·00	293
25	28	F	96	1	0·89	304
26	27	F	96	2	0·50	NR
27	30	M	91	2	0·13	NR
28	54	M	69	2	0·12	NR
29	26	M	109	1	14·5	NR
Mean	29·62		93·64		*0·77	
SEM	2·58		3·90			

*Geometric mean.
$PC_{20}(M)$—concentration of methacholine producing a 20% fall in the baseline FEV_1; PD_{20}(3·6%NaCl)—volume of hypertonic saline laden air required to produce 20% fall in the baseline FEV_1; NR—non-responsive; FEV_1—forced expiratory volume in one second; EIA—exercise induced asthma.

as the first baseline. They were then asked to breathe at tidal volume through the mouthpiece, with the nebuliser switched off, until 25 l of air had been respired. Two further estimations of FEV_1 were made, and the higher reading was taken as the new baseline. If the new baseline differed from the first by more than 10%, the procedure was discontinued, although this was not necessary for any of the patients during the study. The nebuliser was then switched on and, wearing a nose clip, the patient was instructed to breathe quietly through the mouthpiece. After he or she had breathed 5 l of hypertonic saline laden air, single recordings of FEV_1 were made at 30, 90, and 180 seconds. If any FEV_1 estimation was technically poor because of coughing, it was repeated after 30 seconds. If the FEV_1 had fallen by over 20% from the new baseline FEV_1 value, the test end point had been reached. If not, a further volume of air was respired and the same procedure repeated. The volumes used were: 5, 10, 15, 20, 20, 25, 35, 40, 40, 50, and 50 l (BTPS), administered in this sequence until a greater than 20% fall in FEV_1 had occurred, or a total volume of 310 l of hypertonic saline laden air had been respired.

The dose of hypertonic saline administered was expressed as the volume of air drawn from the nebuliser and plotted on a linear scale against the percentage fall in FEV_1. The provocative volume of hypertonic saline laden air required to induce a 20% fall in FEV_1 (PD_{20}) was calculated by linear interpolation.

DATA ANALYSIS

The patients were randomised according to random number allocation. They were ranked according to their response to the hypertonic saline, histamine and methacholine challenge, and the exercise induced asthma symptom severity score. The degrees of correlation between the PD_{20} hypertonic saline, the PC_{20} histamine and methacholine, and the exercise induced asthma symptom score were examined by Spearman's rank correlation. The correlations were made with the Spearman's correlation coefficient because we were able to include all patients in the analysis by ranking those who did not respond to hypertonic saline (Nos 14, 15, 27, 28, and 29) equal last. A level of $p<0·05$ was taken as significant.

Results

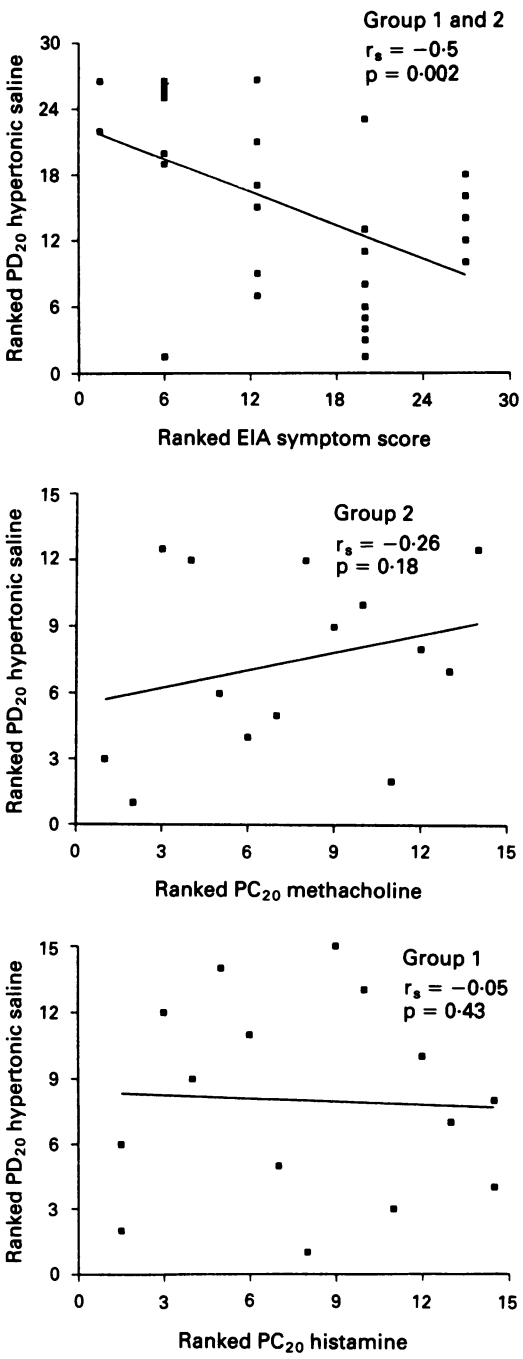
Hypertonic saline produced bronchoconstriction amounting to a reduction of at least 20% of the baseline FEV_1 in 23 (79%) of the 29 patients; PD_{20} values varied from 91 to 310 l (figure). A highly significant correlation was found between the response to hypertonic saline measured as the PD_{20} and the exercise induced asthma symptom score ($r_S=0·5$; $p=0·002$).

GROUP 1

The histamine PC_{20} values ranged from 0·19 to 13·7 mg/ml (geometric mean 1·16 mg/ml). Thirteen of the 15 subjects (87%) responded with a fall in FEV_1 of 20% or more with hypertonic saline challenge (PD_{20} range 9–295 l). The correlation between PD_{20} hypertonic saline and exercise induced asthma symptom score ($r_S=0·27$, $p=0·15$) did not reach significance. The correlation between PD_{20} hypertonic saline and PC_{20} histamine was not significant ($r_S=0·05$, $p=0·43$). No correlation could be found between the exercise induced asthma symptom score and PC_{20} histamine reactivity ($r=0·17$, $p=0·26$).

GROUP 2

The methacholine PC_{20} ranged from 0·04 to 14·5 mg/ml (geometric mean 0·77 mg/ml). Ten of the 14 subjects (71%) responded to the hypertonic saline (PD_{20} range 25–310 l). There was a significant correlation between hypertonic saline PD_{20} and exercise induced asthma symptom score ($r_S=0·62$, $p=0·008$) in this group. No correlation could be established between PD_{20} hypertonic saline response and PC_{20} methacholine (r_S 0·26, $p=0·18$). On removal of the subjects who did not respond to hypertonic saline, however, there was a trend towards a significant relationship between hypertonic saline and methacholine responsiveness ($r_S=0·52$,



The Spearman's ranked correlation (r_S) between the response to hypertonic saline and exercise induced asthma (EIA) symptom score (top, groups 1 and 2), methacholine reactivity (middle, group 2), and histamine reactivity (bottom, group 1). The hypertonic saline response was ranked from most responsive (lowest PD_{20}) to non-responsive (PD_{20}>310 l), histamine/methacholine from lowest PC_{20} to highest PC_{20}, and EIA symptom score from no symptoms (lowest score) to severe symptoms (highest score).

p=0·05). No significant relation could be found between the exercise induced asthma symptom score and the methacholine reactivity (r_S=0·12, p=0·33).

Discussion

In this study we failed to find any significant relationship between the level of airway responsiveness to hypertonic saline and non-specific bronchial hyperresponsiveness measured with histamine or methacholine. A similar result has also been shown by Boulet *et al*,[4] who used a quantitative method to measure the hypertonic saline response with doubling concentrations of saline from 0·9% to 14·4% to obtain a dose-response curve, and expressed the level of hypertonic responsiveness as the osmolarity causing a 20% fall in FEV_1 (PD_{20}) in asthmatic subjects with methacholine PC_{20} values of 0·19–5·54 mg/ml. Belcher *et al*[6] reported a significant correlation between the hypertonic saline and the histamine reactivity in asthmatic subjects responsive to hypertonic saline who had moderate histamine reactivity (geometric mean PC_{20} histamine, 1·08 mg/ml). Smith *et al*[5] have also reported a significant correlation between the hypertonic response and the methacholine reactivity; in this study, however, the subjects had moderate to severe methacholine responsiveness (geometric mean PC_{20} methacholine, 0·68 μmol). In the present study, exclusion of those subjects whose airways failed to respond to hypertonic saline improved the correlation with methacholine PC_{20}, which suggests that in this subgroup the severity of underlying "non-specific" hyperresponsiveness may bear some relation to the degree of hypertonic response. The failure of this study to detect a relationship between bronchial hyperresponsiveness and hypertonic saline response may be due to the relatively small number of subjects used in each part of the study. Such a study would therefore only be likely to detect a strong relationship and the possibility that a weaker relationship does exist has not been excluded, nor has a relationship applying only to a subgroup of subjects such as those with severe bronchial hyperresponsiveness.

The exact mechanisms of hypertonic saline induced bronchoconstriction are not known. In the knowledge that selective H_1 receptor antagonism removes the majority of the response,[14] one suggested mechanism might involve the release of mediators from mucosal mast cells.[15] Anticholinergic drugs also offer some variable degrees of protection, possibly implicating a neuronal reflex mechanism as a result of the loss of epithelial integrity.[15] How much these mechanisms contribute to the overall bronchoconstrictor response is not known, but contributions are likely to vary from subject to subject as with exercise induced asthma. Bronchoconstriction provoked by the cholinergic agonist methacholine occurs by direct interaction with the muscarinic M_3 receptors linked to excitation-contraction coupling of airway smooth muscle.[16] In the case of histamine, the constrictor response is due to a combination of smooth muscle contraction both directly and indirectly through neural reflexes and microvascular leakage, all involving H_1 receptors.[16] In view of the different mechanisms of action of hypertonic saline, histamine, and methacholine in evoking bronchoconstriction, it is not surprising that a relationship between the former probably indirect and the latter more direct stimuli could not be found.

In previous studies severity of asthma has been assessed in terms of symptoms and other features of the history, degree of airflow obstruction, and level of treatment needed to control symptoms. Each of these has its imitations.[17] While measurements of FEV_1 and peak expiratory flow rate (PEF) are both invaluable in assessing the degree and variability of obstruction of airflow, reduction

in airway calibre may occur over short periods of time and is not revealed by intermittent measurements. Bronchial hyperresponsiveness is a functional abnormality and might reflect underlying pathological processes in the airway rather than a relationship with the current degree of bronchoconstriction measured at any one time. Makino[10] and Murray *et al*[11] have reported a stronger correlation between the asthma history score and PC_{20} histamine than between asthma score and any spirometric test in asthmatic subjects who were apparently free of airway obstruction at the time of testing. Similarly, exercise induced bronchoconstriction has been shown to correlate with wheezing score, but not with pre-exercise PEF.[12]

In the present study we have shown a relationship between the exercise induced asthma symptom score and bronchial responsiveness to hypertonic saline, histamine, and methacholine. The close relationship observed between the exercise induced asthma symptom score and the hypertonic saline response confirms the findings of Belcher *et al*,[6] who showed a significant correlation between the level of hypertonic responsiveness and exercise induced bronchoconstriction. The failure of our study to show an association between a symptom score for exercise induced asthma and both histamine and methacholine responses adds to the view that exercise induced asthma is a more complex expression of airway hyperresponsiveness than the simple measurement of histamine or methacholine PC_{20}. While there are studies showing a correlation between exercise induced asthma and histamine and methacholine reactivity, these have always been conducted on selected patient populations.[7 8] When subjects were selected from the community on the basis of respiratory symptoms alone, the degree of bronchoconstriction provoked by exercise failed to relate to methacholine responsiveness.[9]

The significant correlation observed between the exercise induced asthma symptom score and hypertonic saline response, compared with the lack of association between the symptom score for exercise induced asthma and both histamine and methacholine response, is probably partly due to the larger number of subjects in the hypertonic saline group. We therefore examined the correlation between exercise induced asthma and hypertonic response in the two subgroups; no significant correlation was found in the histamine subgroup (group 1), but in the methacholine subgroup the correlation was still significant (group 2). However, in both subgroups the relationship between exercise induced asthma symptom score and hypertonic saline was closer than the relationship between exercise induced asthma symptom score and both histamine and methacholine response. Further studies with larger numbers of patients are needed to confirm these findings.

The response rate of 79% to hypertonic saline challenge in subjects with a history of exercise induced asthma is similar to that reported in other studies.[2-5] Two possible mechanisms have been advanced for the pathogenesis of exercise induced asthma. Smith and Anderson have presented a strong argument that the hypertonicity of the airway lining fluid resulting from conditioning of inspired air to body temperature and humidity is a sufficient stimulus to account for the release of mediator from mucosal mast cells and bronchoconstrictor response to exercise.[15] This argument is supported by the facts that H_1 histamine antagonists are highly effective in inhibiting the airways response to exercise[18] and that a leukotriene receptor antagonist markedly attenuates exercise induced bronchoconstriction.[19] McFadden *et al* have suggested an alternative mechanism from detailed studies of temperature gradients down the airway during exercise induced asthma, and hypothesised that airway cooling followed by rebound hyperaemia might be responsible for the reduction in airway calibre.[20] Our finding that the perception of severity of exercise induced asthma by patients relates closely to the level of airway responsiveness to hypertonic aerosol challenge supports a link between hypertonicity and the mechanisms of exercise induced asthma. That the two types of challenge share a common pathway is further supported by cross refractoriness between the two stimuli.[21]

We therefore conclude that airway responsiveness to hypertonic saline relates more closely to the symptom severity of exercise induced asthma than to histamine or methacholine reactivity. These findings reinforce the view that hypertonic saline induced bronchoconstriction is a challenge model that closely reflects the mechanisms of exercise induced asthma.

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