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Sodium cromoglycate and atropine block the fall in FEV_1 but not the cough induced by hypotonic mist

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ABSTRACT In a group of patients with mild asthma the inhalation of mist derived from ultrasonically nebulised distilled water caused an increase in cough and a fall in FEV_1. Double blind administration for five minutes of sodium cromoglycate (from an original solution containing 30 mg/ml) or atropine (2 mg/ml) by inhalation from a Minineb nebuliser, 30 minutes before the mist challenge, caused a significant reduction in the fall in FEV_1 ($p < 0.05$), but not in cough, by comparison with the protection afforded by placebo (saline). In a second study the fall in FEV_1 caused by the inhalation of distilled water was not significantly different from that seen in response to hypotonic sodium chloride (1·7 g/l, 58 mmol/l), but both produced a significantly greater fall than did a similar mist containing sodium cromoglycate at an original concentration of 10 mg/ml (58 mmol/l). The results show that both atropine and sodium cromoglycate can block the fall in FEV_1 due to mist and that protection by sodium cromoglycate is immediate. These results suggest that sodium cromoglycate blocks the nervous reflexes concerned in the response to mist, probably in the afferent limb of the reflex.

Patients with asthma regularly develop increased airways resistance after inhaling mist derived from ultrasonically nebulised hypotonic solutions,[1–4] and the response in an individual correlates well with the change that follows a non-specific bronchoconstrictor challenge such as exercise.[2] The response to mist depends on the dose given and on the osmolarity of the original solution; hypertonic and hypotonic solutions cause bronchoconstriction while normal saline has no effect.[1 3 4] Results of animal studies suggest that the response is reflex and secondary to stimulation of receptors sensitive to changes in osmolarity.[5 6] Such a response should be amenable to modification by drugs that interfere with autonomic nervous control, but previous attempts to use drugs to influence the response[1 4 7] have been inadequately controlled and the results inconclusive.

We report here two placebo controlled studies designed to assess whether drugs can interfere with the airway response to hypotonic solutions. The first, a double blind study, tested whether pretreatment with sodium cromoglycate, atropine, or placebo modified the effect on FEV_1 of inhaled mist derived from distilled water. The second, which was single blind, assessed the speed of onset of the effect of sodium cromoglycate.

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Methods

Studies were performed with the patients seated in a comfortable, quiet laboratory. The ambient temperature varied from 21°C to 24°C. Subjects gave informed consent, and the study had the approval of the local ethical committee. Six asthmatic patients (mean age 56 years), with a mean forced expiratory volume in one second (FEV_1) of 73% of the predicted normal, took part in the first study. All regularly used inhalations of beclomethasone and a β_2 sympathomimetic and four were maintained on oral prednisolone (<7·5 mg/day). None was taking sodium cromoglycate or atropine like drugs and β sympathomimetics were omitted for 12 hours before each study.

The subjects were studied on five separate occasions at the same time of day with at least three days separating each attendance. Separation of studies by several days is important since observations in this and other laboratories have shown that if the mist challenge is repeated within 24 hours desensitisation develops and this would complicate interpretation of any response to drugs. On each day a "prechal-

lenge" measurement of FEV_1 was made with a dry wedge spirometer (Vitalograph) and the subjects were then challenged with an inhalation of mist derived from distilled water. The figure taken for the prechallenge FEV_1 was the highest value recorded in three manoeuvres; only a single manoeuvre was used to measure FEV_1 on subsequent occasions.

The mist was produced by a Devilbiss 65 ultrasonic nebuliser set to deliver 6 ml min^{-1} of mist with a wide range of particle size but with 15·5% having a diameter less than 2 μ.[4] The subjects breathed normally, inhaling the mist for five minutes through a two way valve. The number of coughs made by the subjects was recorded during the period of inhalation. FEV_1 was recorded at 1, 3, 5, 10, 15, 20, and 30 minutes after the end of the challenge. At the end of this 30 minute period a bronchodilator was given if the FEV_1 was less than 90% of the prechallenge level.

On the first and last study day mist challenge alone was given; on the three intermediate days subjects were pretreated 30 minutes before the challenge with either sodium cromoglycate (from an original solution containing 30 mg/ml), atropine methonitrate (2 mg/ml), or saline (0·9%), each inhaled for five minutes from a Minineb nebuliser driven by air at 10 lb/in^2 (69 kPa). The order in which the drugs was given was randomised and double blind. On the days when the subjects received drugs a "baseline" measurement of FEV_1 was made before treatment at approximately 30 minutes before the prechallenge recordings. Blood was taken immediately before and 30 minutes after the challenge with mist and assayed by radioimmunoassay[8] for sodium cromoglycate and by receptor binding assay for atropine. The dose of sodium cromoglycate reaching the lungs was estimated from the plasma concentrations and the subject's weight.[8]

In the second study, designed to assess the speed of onset of the effect of sodium cromoglycate, five different subjects (mean age 21 years), all with a history of mild asthma (mean FEV_1 101% of predicted), took part. None was receiving regular medication but each occasionally used a β sympathomimetic, which was withheld for at least 12 hours before each study. The subjects were tested on three different days separated by at least one week. On each occasion a baseline recording of FEV_1 was made and the subject was then challenged with ultrasonically produced mist inhaled via a two way valve for five minutes. On the first day the subject received nebulised distilled water and on subsequent study days challenge was performed single blind, in random order, with a hypotonic mist generated from either sodium cromoglycate (10 mg/ml) or saline (1·7 g/l), both solutions having an osmolarity of 58 mmol/l. The number of coughs during inhalation and the FEV_1 one, three, five, 10, 15, 20, and 30 minutes after the challenge were recorded.

Changes in the FEV_1 were analysed by paired t test, and changes in number of coughs by the sign test. The results are given as means with standard deviations in parentheses.

Results

Effect of pretreatment with sodium cromoglycate or atropine on the response to inhalation of distilled water mist

The results of the first study are shown in table 1. For each subject the "prechallenge" FEV_1 on the five study days was similar, and basal FEV_1 was not altered significantly by treatment with either sodium cromoglycate or atropine. The fall in FEV_1 in response to challenge with nebulised distilled water was maximal at one minute after the challenge and was similar on the two control days (−43% (9%) and −40% (18%)) and on the placebo treatment days (−43% (11%)). The mist induced fall in FEV_1 was significantly attenuated ($<0·05$) both by sodium cromoglycate and by atropine by comparison with the response after placebo (table 1). The cough response was not altered by treatment with either drug.

The mean plasma concentration of sodium cromoglycate at 30 minutes was 6·2 (2·47) ng/ml and at 60 minutes 4·37 (1·98) ng/ml. The plasma concentration of atropine was 0·33 (0·12) mmol/l at 30 minutes and 0·25 (0·10) mmol/l at 60 minutes. From these values of sodium cromoglycate concentration the estimated dose reaching the lungs was 0·7 (0·24) mg.

Table 1 *Baseline, prechallenge, and maximum changes in FEV_1 and number of coughs after mist challenge in six subjects with and without pretreatment with placebo, atropine or sodium cromoglycate (SCG)*

	Control 1	*Placebo*	*Atropine*	*SCG*	*Control 2*
Baseline FEV_1 (l): mean (1 SD)		1·72 (1·08)	1·68 (1·09)	1·68 (1·09)	
Prechallenge FEV_1 (l): mean (1 SD)	1·81 (1·15)	1·88 (1·15)	1·78 (1·05)	1·964 (1·10)	1·77 (1·06)
Maximum % change in FEV_1: mean (1 SD)	−43 (9)	−43 (11)	−18 (22)*	−6 (18)**	−40 (18)
No of coughs: median (range)	10 (3–29)	26 (0–32)	15 (0–19)	14 (4–24)	12·5 (0–32)

*$p < 0·05$; **$p < 0·01$ by comparison with placebo.

Table 2 *Baseline and percentage change in FEV_1 and number of coughs in five subjects after challenge by mist of distilled water or hypotonic solutions of sodium cromoglycate (SCG) or saline*

	Water	SCG	Saline
Baseline FEV_1 (l): mean (+1 SD)	4·37 (0·72)	4·19 (0·66)	4·14 (0·61)
Maximum % change in FEV_1: mean (+1 SD)	−37·4 (14)	−6·4 (5·7)**†	−30·4 (12·1)
No of coughs: median (range)	11 (4–20)	0 (0–9)*	11 (0–38)

*p < 0·05; **p < 0·01 by comparison with water.
†p < 0·05 by comparison with saline.

Effect on FEV_1 and cough of inhaling mists derived from nebulised distilled water, saline, and sodium cromoglycate

The results of the second study are shown in table 2. No difference was found in the baseline FEV_1 on the three study days. The fall in FEV_1 after challenge with distilled water was −37·4% (14%) and after saline (1·7 g/l) −30·4% (12·1%); these figures were not significantly different. After challenge with sodium cromoglycate (10 mg/ml), however, the fall in FEV_1 was significantly less at −6·4% (5·7%) ($p < 0·05$). The number of coughs that occurred was significantly greater during the inhalation of water than during sodium cromoglycate inhalation ($p < 0·05$, sign test). The number was also greater during inhalation of saline than of sodium cromoglycate but this difference did not reach significance. There was no difference between the numbers of coughs during water and saline inhalation.

Discussion

Previous workers have shown that the inhalation of a mist produced by ultrasonically nebulised distilled water causes cough but no change in airway tone in normal subjects, and cough and a reproducible increase in airway tone in patients with asthma.[1 3 4] The results of the first part of the present investigation essentially confirmed these observations in that in our experiments a five minute inhalation by asthmatics of a mist derived from distilled water produced a fall in FEV_1 of over 15% and also cough. In addition, however, these studies show that both sodium cromoglycate and atropine, given by nebuliser 30 minutes before the mist challenge, significantly reduced the fall in FEV_1. This is therefore similar to the effects of these drugs on challenges with exercise and inhalation of cold air.[9 10] Studies using atropine are often difficult to interpret because the drug may cause a change in baseline measurements. In this investigation we have used a dose of atropine that we had shown in earlier studies in healthy volunteers and people with mild asthma did not alter basal FEV_1. The protection afforded by atropine is therefore unlikely to be an artefact secondary to a change in basal tone. The cough response to the inhalation of hypotonic mist was poorly reproducible (table 1); even so there was no indication of an inhibition of the response by the prior inhalation of either sodium cromoglycate or atropine.

The second series of experiments, using a hypotonic solution of sodium cromoglycate, showed that the fall in FEV_1 that occurred when mist was inhaled was blocked by the presence of sodium cromoglycate. The possibility that there was a reduced response because the sodium cromoglycate solution had a greater osmolarity than distilled water cannot be sustained, since a solution of saline of similar osmolarity to the cromoglycate solution produced a fall similar to that of inhaled water. Cromoglycate does not therefore appear to be needed at the site of action before the challenge for the response to be blocked. In this second study the inhalation of cromoglycate caused significantly less coughing than distilled water but not less than saline; this may be explained by the fact that the osmolarity of the cromoglycate solution is higher than that of distilled water and would be in keeping with the findings of Cheney and Butler,[11] who also found that half normal saline produced less coughing than did water.

Animal studies have shown that the direct application of hypotonic solutions to the upper airways produces increased activity in the sensory nerves supplying the mucosa[5 6] and produces airways constriction.[12] Both Boushey *et al*[5] and Harding *et al*[6] found that there were two different groups of receptors that responded to a hypotonic challenge, and Colebatch and Halmagyi[12] concluded that the cough and airway changes were caused by different nervous reflexes. Our studies support the hypothesis that there are two different reflex arcs, one causing cough and the other a change in FEV_1, the two being influenced differently by drugs.

Blockade of the bronchoconstrictor response to mist by atropine, which has also been reported by Sheppard *et al*,[4] offers strong evidence that the response is mediated by a nervous mechanism as in animals.[12] The time course of the response to mist, with the maximal fall in FEV_1 occurring immediately after the challenge, also favours a response that is mediated through a nervous pathway rather than due to release of mediators from inflammatory cells, as with antigen challenge, a mechanism that tends to take rather longer to act. Release of specific mast cell mediators has not been reported after chal-

lenge with mist and, although raised concentrations of histamine have been reported in exercise induced asthma by some workers,[13] this has not been found by others.[14] A confounding problem in these studies has been that the changes in plasma mediator concentration might have been due to changes in circulating blood basophils.[14 15] The recently reported rise in plasma neutrophil chemotactic factor after mist challenge[16] might therefore also be secondary to changes in circulating cells,[17] and the rise does not appear to be related specifically to events in the asthmatic lung since it also occurs with pulmonary infection.[18] If the response to mist were due to mediator release, then challenge might produce a "late phase" as is seen after inhalation of antigen. No such response has been reported and none was experienced by any of the subjects in this study, even though one was known to show a late response to antigen.

The mechanism by which sodium cromoglycate inhibits the response to mist is uncertain. Direct evidence for cromoglycate induced inhibition of mediator release from mast cells in man has been difficult to find[19] and animal studies suggest that the response to mist is secondary to stimulation of afferent nerves, rather than due to release of mediators from inflammatory cells. It therefore seems likely that cromoglycate is acting by interfering in some way with a nervous reflex. Certainly the immediacy of the effect of cromoglycate on the mist induced fall in FEV_1 in this study favours inhibition of such a reflex by cromoglycate as the mode of action. The likely site of action would be at the sensory receptor, as has been reported for the bronchoconstrictor reflex in dogs arising from left ventricular receptors[20] and airway C fibres.[21] The concentration of sodium cromoglycate in plasma at the time of blockade of the effect of the mist challenge was about 6·5 ng/ml. Interestingly, a threefold greater concentration is required to inhibit the activity of mast cells taken from rat peritoneum[22] or isolated from human lung,[23] and even these high concentrations do not affect the activity of mast cells in human skin.[19] The concentration in the airways, although initially high, is likely to have fallen below the equivalent in vitro concentrations, as the dose received by the subject (about 0·7 mg) will have been distributed throughout the airways and some will have been absorbed in the 30 minutes before the challenge. Any inhibition of sensory nerves could not have been uniform as the cough response was not blocked. Cromoglycate has previously been shown not to modify cough produced by C fibre stimulation in man.[24]

The present study therefore suggests that the inhalation of hypotonic mist in man causes a fall in FEV_1 and cough by the stimulation of two separate nervous reflexes. The bronchoconstrictor reflex is inhibited by both sodium cromoglycate and atropine. It is postulated that these drugs act by inhibiting either the afferent (cromoglycate sensitive) or efferent (atropine sensitive) limbs of the reflex.

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References

1 Anderson SD, Schoeffel RE, Finney M. Evaluation of ultrasoncially nebulised solutions for provocation testing in patients with asthma. *Thorax* 1983;**38**:284–91.

2 Higenbottam T, Stokes TC, Jamieson S, Hill L. Bronchial reactivity in asthmatic adults with normal spirometric values. *Br Med J* 1983;**286**:1012–4.

3 Schoeffel RE, Anderson SD, Altounyan RE. Bronchial hyperreactivity in response to inhalation of ultrasonically nebulised solutions of distilled water and saline. *Br Med J* 1981;**283**:1285–7.

4 Sheppard D, Rizk NA, Boushey HA, Bethel RA. Mechanism of cough and bronchoconstriction induced by distilled water aerosol. *Am Rev Respir Dis* 1983;**127**:691–4.

5 Boushey HA, Richardson PS, Widdicombe JG, Wise JCM. The response of laryngeal afferent fibres to mechanical and chemical stimuli. *J Physiol* 1974;**240**:153–75.

6 Harding R, Johnson P, McClelland ME. Liquid-sensitive laryngeal receptors in the developing sheep, cat and monkey. *J Physiol* 1978;**277**:409–22.

7 Allegra L, Bianco S. Non-specific broncho-reactivity obtained with ultrasonic aerosol of distilled water. *Eur J Respir Dis* 1980;**61**:41–9.

8 Fuller RW, Collier JG. The pharmokinetic assessment of sodium cromoglycate. *J Pharmacol* 1983;**35**:289–92.

9 Eggleston PA, Bierman W, Pierson WE, Stamm SJ, Van Arsdal PP. A double-blind trial of the effect of cromolyn sodium on exercise-induced bronchospasm. *J Allergy Clin Immunol* 1972;**50**:57–62.

10 Breslin FD, McFadden ER, Ingram RH. The effect of cromolyn sodium on the airway response to hyperpnea and cold air in asthma. *Am Rev Respir Dis* 1980;**122**:11–6.

11 Cheney FW, Butler J. The effects of ultrasonically produced aerosols on airway resistance in man. *Anaesthesiology* 1968;**29**:1099–106.

12 Colebatch HJH, Halmagyi DFJ. Reflex airway reaction to fluid aspiration. *J Appl Physiol* 1962;**17**:787–94.

13 Lee TH, Brown MJ, Nagy L, Causon R, Walport MJ, Kay AB. Exercise-induced release of histamine and neutrophil chemotactic factor in atopic asthmatics. *J Allergy Clin Immunol* 1982;**70**:73–81.

14 Morgan DJR, Moodley I, Phillips MJ, Davies RJ. Plasma histamine in asthmatic and control subjects following exercise: influence of circulating basophils and different assay techniques. *Thorax* 1983;**38**:771–7.

15 Howarth PH, Holgate ST. Exercise, asthma and mast cells. *Lancet* 1983;i:822.

16 Shaw R, Durham S, Torzillo P, *et al*. Neutrophil

chemotactic activity following the inhalation of ultrasonically nebulised water. *Aust NZ J Med* 1983; **13**:541.

[17] Cundell DR, Morgan DJR, Davies RJ. NCF—a mast cell specific chemotactic factor? *Clin Sci* 1984;**66**:50P (abstract).

[18] Lee TH, Nagakura T, Papageorgiou N, Ikura Y, Kay AB. Exercise-induced late asthmatic reactions with neutrophil chemotactic activity. *N Engl J Med* 1983;**308**:1502–5.

[19] Phillips MJ, Meyrick TRH, Moodley I, Davies RJ. A comparison of the in vivo effect of ketotifen, clemastine, chlorpheniramine and sodium cromoglycate on histamine and allergen induced weals in human skin. *Br J Clin Pharmacol* 1983;**15**:277–86.

[20] Dixon M, Jackson DM, Richards IM. The effects of sodium cromoglycate on lung irritant receptors and left ventricular cardiac receptors in the anaesthetised dog. *Br J Pharmacol* 1979;**67**:569–74.

[21] Dixon M, Jackson DM, Richards IM. The action of sodium cromoglycate on 'C' fibre endings in the dog lung. *Br J Pharmacol* 1980;**70**:11–3.

[22] Ennis M, Trunem A, White JR, Pearce FL. Inhibition of histamine secretion from mast cells. *Nature* 1981;**289**:186–7.

[23] Church MK, Holgate ST, Pao GJK. Histamine release from mechanically and enzymatically dispersed human lung mast cells: inhibition by salbutamol and cromoglycate. *Br J Pharmacol* 1983;**79**:374P (abstract).

[24] Collier JG, Fuller RW. Capsaicin inhalation in man and the effects of sodium cromoglycate. *Br J Pharmacol* 1984;**81**:113–7.