Inhibition by sodium cromoglycate of bronchoconstriction stimulated by respiratory heat loss: comparison of pressurised aerosol and powder

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ABSTRACT The protective effect was examined of three doses (2, 10, and 20 mg) of sodium cromoglycate inhaled from a pressurised metered dose inhaler on the response to isocapnic hyperventilation of cold dry air in 10 asthmatic subjects. This was compared with the effect of cromoglycate powder (20 mg) inhaled from a Spincap and with placebo given on two occasions. The medications were inhaled on separate days, in random order and with the use of a double blind double dummy technique, 20 minutes before isocapnic hyperventilation of two fold increasing volumes of air (-15°C , 0% humidity) to produce a 20% fall in the post-treatment FEV_1 . The response was expressed as the provocative dose of respiratory heat loss required to cause a fall in FEV_1 of 15% (PD_{15} , kcal/min). The mean baseline spirometric indices exceeded 85% of predicted normal values on each test day; both placebo treatments reduced the baseline FEV_1 by comparison with all active treatments ($p < 0.0001$). Comparison of the PD_{15} on the two placebo days confirmed excellent reproducibility. All doses of cromoglycate shifted the respiratory heat loss dose-response curve to the right of the placebo curve; PD_{15} after all active treatments exceeded PD_{15} after placebo ($p < 0.0001$). There was no cromoglycate dose-response relationship between the three doses of aerosol ($p > 0.05$), or between any dose of aerosol and powder ($p > 0.05$). It is concluded that cromoglycate aerosol inhaled from a pressurised inhaler in a dose of 2 mg gives the same magnitude of protection against bronchoconstriction stimulated by airway cooling as 20 mg of pressurised aerosol or powder from a Spincap.

Pretreatment of the asthmatic airway with inhaled sodium cromoglycate as dry powder, a nebulised solution, and pressurised aerosol can reduce the bronchoconstrictor response to exercise.¹⁻⁵ Similar inhibition has been demonstrated after inhalation of cromoglycate solution (20 mg) and powder (20 mg),³ and after powder (20 mg) and pressurised aerosol (2 mg).⁵ The most effective dose of pressurised aerosol has not been established.

Recent work by McFadden and coworkers has identified the importance of airway cooling in the development of exercise stimulated bronchoconstriction and has introduced a thermal challenge using maximal ventilation of cold dry air.⁶⁻⁹ We have modified their method to allow the construc-

tion of respiratory heat loss dose-response curves and have used it to examine the effect of drugs on bronchoconstriction stimulated by respiratory heat loss.¹⁰⁻¹³

In this study we compared the protective effect of three doses of cromoglycate aerosol (2, 10, and 20 mg) with powder (20 mg) and placebo on the response to respiratory heat loss in 10 adults with asthma, to identify the most effective dose of aerosol.

Methods

Ten adult asthmatics attending the Firestone regional chest and allergy unit participated in this study (table 1). All had a history of episodic wheeze and dyspnoea and described symptoms of exercise stimulated bronchoconstriction. All were atopic as shown by weal and flare responses to skin prick tests with

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Table 1 Characteristics of the subjects

Subject No	Age (y)	Sex	Height (cm)	Atopy*	Current treatment†
1	32	M	171	6	S not daily
2	19	M	183	6	S not daily
3	25	M	173	8	S 200, T 200
4	29	F	162	4	S 1000, T 900, B 400
5	26	F	163	4	F 800, B 200
6	22	M	173	7	S 400, T 600, B 200
7	35	M	179	6	S 800
8	29	M	188	6	F not daily
9	25	F	166	4	S 400, B 200
10	25	F	171	5	S 400

*Number of positive weal and flare responses to skinprick tests with 16 common allergen extracts.

†S—salbutamol (μg); B—beclomethasone dipropionate (μg); F—fenoterol (total dose per day, μg); T—slow release theophylline (total dose per day, mg).

16 common allergen extracts. Over the period of the study, symptoms of asthma did not disturb sleep and the baseline FEV_1 before delivery of any test medication exceeded 70% of predicted normal. No subject had experienced symptoms of respiratory infection or been exposed to a sensitising allergen (with the exception of regular exposure to house dust in subjects 3 and 5–10) in the month preceding the study. The study was approved by the hospital research committee and each subject gave written informed consent.

Each subject attended the laboratory at the same time of day on six test days within three weeks. Aerosol bronchodilator treatment was withheld for six hours and theophylline for 36 hours, but the use of beclomethasone was not interrupted; no subject used cromoglycate for control of asthma. After a 15 minute rest three measurements of FEV_1 spaced one minute apart were made with a 9 litre Collins water spirometer. The test medications were then given double blind in random order. They were: 1—placebo aerosol (four puffs), placebo powder; 2—placebo aerosol (four puffs), placebo powder; 3—cromoglycate aerosol (two puffs, 1 mg/puff), placebo aerosol (2 puffs), placebo powder; 4—cromoglycate aerosol (two puffs, 5 mg/puff), placebo aerosol (two puffs), placebo powder; 5—cromoglycate aerosol (4 puffs, 5 mg/puff), placebo powder; 6—placebo aerosol (four puffs), cromoglycate powder (20 mg). Aerosols were inhaled from pressurised metered dose canisters and powder from a capsule (Spinhaler) placed in an inhaling device (Spinhaler). All medications were given in a standardised manner. The subject exhaled to near functional residual capacity (FRC), and the pressurised canister was positioned 3 cm from the wide open mouth and activated once by the experimenter just after the beginning of a slow inhalation to near total lung capacity (TLC). The subject then held his breath for 10 seconds. The remaining three inhalations were delivered consecutively by the same

technique. Finally, the subject exhaled to near FRC and, holding the Spinhaler between the lips, quickly inhaled to near TLC and again held his breath for 10 seconds. Twenty minutes later the FEV_1 was remeasured three times, and isocapnic hyperventilation of subfreezing dry air was begun.

Isocapnic hyperventilation was carried out by the method used by O'Byrne *et al.*¹⁰ which was a modification of that described by Strauss *et al.*⁷ Cold dry air (-15°C , 0% relative humidity), generated by a heat exchanger, was inhaled in twofold increasing volumes (7.5, 15, 30, and 60 l/min and at maximum voluntary ventilation) for three minutes at intervals of five minutes. Carbon dioxide was added to the inhaled air to keep the subject eucapnic during each period of hyperventilation. The response was measured by the change in FEV_1 from the lowest post-treatment value to the lowest value recorded 0.5 and 1.5 minutes after each inhalation. If there was a fall in FEV_1 the measurement was repeated at three minutes and at subsequent two minute intervals until the lowest value was recorded. Once the FEV_1 started to improve the subject received the next inhalation of cold air. Inhalations were discontinued once the FEV_1 had fallen by 20% or after maximum voluntary ventilation. The final level of hyperventilation was completed no later than 70 minutes after delivery of the pretreatment for all subjects on all test days. The respiratory heat loss (RHL, kcal/min) was calculated for each level of ventilation from the formula $\text{RHL} = \dot{V}_E (\text{HC} [\text{Ti} - \text{Te}] + \text{HV} [\text{WC}_i - \text{WC}_e])$, where $\dot{V}_E$ = minute ventilation (l/min), HC = heat capacity of air (0.000304 kcal/min), Ti = inspired air temperature ($^\circ\text{C}$), Te = expired air temperature ($^\circ\text{C}$), HV = latent heat of vaporisation of water (0.00058 kcal/mg), WC_i = water content of inspired air (mg/l), and WC_e = water content of expired air (mg/l). Fast responding thermistors placed within the mouthpiece recorded Ti and Te . The inspired air was dry. Expired air was assumed to be fully

saturated at the expired temperature,¹⁴ and the water content was obtained from standard saturation temperature relationships.¹⁵ For each test condition dose-response curves were constructed with FEV₁ (percentage change from the lowest post-treatment value) on the ordinate and respiratory heat loss (kcal/min) expressed logarithmically on the abscissa. The provocative concentration of respiratory heat loss causing a 15% fall in FEV₁ (PD₁₅, kcal/min) was obtained from the log dose-response curves by linear interpolation of the points above and below the 15% fall in FEV₁.

In this experiment each subject was studied on six occasions, twice after placebo and four times after active treatment, in random order. The data for each outcome, percentage change in FEV₁ after treatment and PD₁₅, therefore formed a randomised block design¹⁶ with the subjects as "blocks." The appropriate approach for comparing treatments was thus two way analysis of variance, which resulted in an F test of the difference between the six treatment means. Rather than conducting a large number of comparisons between pairs of treatments, the technique of linear contrasts¹⁷ was used (a) to compare the mean after placebo with the mean after each active treatment, (b) to test the degree of any treatment dose-response correlation within the three doses of aerosol, and (c) to compare the effect of the 20 mg aerosol and powder treatments. Analysis of covariance¹⁸ was used to determine whether the observed treatment effects on PD₁₅ could be explained solely by a change in airway calibre.

The reliability of PD₁₅ was examined by estimation of the within subject and between subject components of variance from a one way analysis of variance of the results from the two tests after placebo.

Reproducibility was quantified by the within subject standard deviation of PD₁₅. The intraclass correlation, the ratio of between subject variance to the sum of between subject plus within subject variance, was used as a reliability coefficient. The intraclass correlation is the proportion of total variance due to real differences between subjects and can be thought of as a signal to noise ratio—or, more exactly, a signal to signal plus noise ratio.¹⁹

Results

The mean pretreatment FEV₁ on the six treatment days showed only slight variation (table 2) that was clearly non-significant ($p = 0.45$). Inhalation of all test medications produced a small reduction in mean FEV₁. This effect was greater after the two placebo treatments (mean 4.7%) than after the four cromoglycate treatments (mean 2.3%). This difference, although small, was highly significant ($p < 0.0001$). There is little evidence of any real differences in mean reduction in FEV₁ after the four individual cromoglycate treatments ($p > 0.05$).

There was a shift to the right in the respiratory heat loss dose-response curves after all cromoglycate treatments by comparison with placebo (fig). The mean PD₁₅ after placebo was 1.62 kcal (6.78 kJ)/min, compared with the mean after all cromoglycate treatments of 1.99 kcal (8.33 kJ)/min ($p < 0.0001$). The mean PD₁₅ values after individual cromoglycate treatments (table 2) were very similar; the test for trend with dose among the aerosol groups showed this to be non-significant ($p > 0.5$), and similarly with the comparison between the 20 mg dose of aerosol and powder ($p > 0.5$). The effect of the post-treatment FEV₁ on PD₁₅ was examined

Table 2 Effects of placebo and sodium cromoglycate on response to respiratory heat loss

Subject No	Placebo			Placebo			Aerosol cromoglycate 2 mg			Aerosol cromoglycate 10 mg			Aerosol cromoglycate 20 mg			Powder cromoglycate 20 mg		
	FEV ₁ (% pred)	%Δ FEV ₁ *	PD ₁₅ †	FEV ₁	%Δ FEV ₁ *	PD ₁₅ †	FEV ₁	%Δ FEV ₁ *	PD ₁₅ †	FEV ₁	%Δ FEV ₁ *	PD ₁₅ †	FEV ₁	%Δ FEV ₁ *	PD ₁₅ †	FEV ₁	%Δ FEV ₁ *	PD ₁₅ †
1	85.2	-5.2	2.04	85.2	-1.8	2.22	85.9	-2.3	2.47	82.7	-2.9	2.33	79.8	-1.5	2.51	78.1	-4.4	>2.53
2	90.1	-5.9	1.92	87.5	0.5	1.81	86.2	3.3	2.54	89.0	-1.6	2.15	88.4	-0.7	2.82	91.7	-2.4	>2.58
3	88.0	-4.4	1.67	83.4	-5.2	1.41	82.1	-1.4	>2.43	82.1	-3.1	2.10	86.4	-3.5	1.98	83.4	-2.7	2.17
4	94.6	-5.1	1.22	94.3	-9.5	1.04	99.5	-9.9	1.20	108.5	-1.7	1.89	99.4	-4.9	1.32	104.0	-4.6	1.43
5	105.4	-4.3	2.11	106.0	-1.9	1.80	103.5	0.6	2.98	107.0	-3.1	>2.70	106.0	0	2.79	103.7	-0.5	2.16
6	73.9	-9.2	1.13	71.7	-0.6	1.16	69.1	-0.6	1.37	70.2	-1.3	1.27	72.3	-5.9	1.09	70.9	-3.2	1.19
7	83.6	-13.2	1.86	86.7	-4.8	2.05	80.8	1.4	2.12	86.8	-2.1	2.71	85.5	-4.8	2.18	84.2	-3.8	2.23
8	73.0	-1.9	1.88	72.1	-1.7	1.91	75.2	-1.4	1.92	72.8	0.3	1.94	72.3	0.5	1.96	74.1	-6.0	1.84
9	107.8	-7.7	1.27	109.2	-4.7	1.13	113.5	0	1.66	109.7	-0.8	1.65	106	-6.3	1.73	110.8	-2.3	1.72
0	83.5	-2.6	1.31	93.3	-5.3	1.33	77.5	-1.0	1.26	80.4	-0.6	1.20	79.8	1.7	1.68	95.2	-6.0	1.56
Mean	88.5	-6.0	1.64	88.9	-3.5	1.59	87.3	-1.1	2.00	88.9	-1.7	1.99	87.6	-2.5	2.01	89.6	-3.6	1.94
D	11.6	3.3	0.37	12.4	3.0	0.42	13.9	3.5	0.62	14.6	1.1	0.52	12.5	2.9	0.58	13.7	1.7	0.47

Percentage change in FEV₁ 20 minutes after medication.

Provocative dose of respiratory heat loss producing a 15% fall in FEV₁, expressed in kcal/min (1 kcal = 4.184 kJ).

% pred—percentage of predicted normal.²⁰

Dose-response curves showing the relationship between respiratory heat loss (RHL) and FEV_1 after pretreatment with placebo (●—●—● and ●—●—●), cromoglycate aerosol (2 mg (○), 10 mg (△), and 20 mg (□)), and cromoglycate powder (20 mg, *). Each point represents the mean for 10 subjects and the bars represent one standard deviation above and below the mean. Conversion: Traditional to SI units—heat: 1 kcal = 4.184 kJ.

by analysis of covariance, the FEV_1 being used as a covariate. This left the mean PD_{15} almost unchanged, so the differences in PD_{15} after placebo and after cromoglycate treatments could not be explained as due to the difference in FEV_1 .

The within subject standard deviation of PD_{15} was 0.12 kcal (0.50 kJ)/min, which indicates that a within subject change of 0.35 kcal (1.46 kJ)/min or more would be unlikely to be due to measurement variability. Furthermore, the intraclass correlation for PD_{15} was 0.91 ($p < 0.0001$), indicating good reliability when measurement variation is compared with true between subject differences.

Discussion

This study is the first to examine the protective effect of sodium cromoglycate aerosol inhaled from a pressurised inhaler on the response to hyperventilation of cold air. The results show that the lower dose of 2 mg is more effective than placebo and is as effective as the higher doses of 10 and 20 mg.

The effectiveness of cromoglycate powder in a

dose of 40 mg on bronchoconstriction stimulated by hyperventilation of cold air has been observed in earlier studies.^{21–23} In the present study 20 mg of powder was found to be as effective as 2 mg of pressurised aerosol. This is similar to the effect on bronchoconstriction stimulated by treadmill running.⁵

The observation that the protective effect of pressurised aerosol was not dose related is at variance with the dose-response effect of cromoglycate nebuliser solution on exercise stimulated bronchoconstriction found by Patel *et al.*²⁴ Patel observed increasing protection when estimated doses of 2.4, 12, and 24 mg were generated continuously by a Wright nebuliser and inhaled by tidal breathing over 5 minutes, and no greater protection after inhalation of 48 mg. Presumably the differences between the two studies are due to differences in the doses or distribution (or both) of cromoglycate deposited in the lung by the different methods of aerosol generation and inhalation. In our study the 2 mg dose of pressurised aerosol might have deposited an effective dose in the lung which was equal to or greater than the third dose of Patel *et al.*²⁴ and therefore a higher dose would not have a greater effect. Alternatively, the different results between the two studies may be due to the different methods used to stimulate bronchoconstriction.

Two conflicting theories are currently held about the pathogenesis of exercise stimulated bronchoconstriction. Deal and coworkers²⁵ concluded from a series of experiments using isocapnic hyperventilation of cold air that the major stimulus for exercise triggered bronchoconstriction was airway cooling without the release of chemical mediators. The alternative theory, proposed by Ben-Dov and his colleagues,²⁶ suggests that exercise stimulated bronchoconstriction is primarily temperature independent and caused by mediator release. Recent findings by Lee and colleagues²⁷ that mediator release does occur with respiratory heat loss, regardless of whether the cooling is achieved by exercise or by hyperventilation, incorporates aspects of both theories and is in keeping with the idea the cromoglycate attenuates bronchoconstriction provoked by both exercise and respiratory heat loss through its mast cell stabilising properties.

In practical terms, the results of this study and that of Bundgaard *et al.*⁵ suggest that bronchoconstriction stimulated by cold air or by exercise will be equally inhibited by 2 mg of cromoglycate aerosol inhaled from a pressurised inhaler and by 20 mg of powder from a SpinCap.

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References

- ¹ Poppius H, Muittari A, Kreuz K-E, Korhonen O, Viljanen A. Exercise asthma and disodium cromoglycate. *Br Med J* 1970;iv:337-9.
- ² Eggleston PA, Bierman CW, Pierson WE, Stamm SJ, Van Arsdel PP jun. A double blind trial of the effect of cromolyn sodium on exercise-induced bronchospasm. *J Allergy Clin Immunol* 1972;50:57-63.
- ³ Dahl R, Henriksen JM. Inhibition of exercise-induced bronchoconstriction by nebulised sodium cromoglycate in patients with bronchial asthma. *Scand J Respir Dis* 1979;60:51-5.
- ⁴ Sanguinetti CM, Gasparini S, Bonifazi F, Vennarucci LS. Exercise-induced asthma diagnosis and prevention with a metered dose aerosol formulation of sodium cromoglycate. *Respiration* 1982;43:132-41.
- ⁵ Bundgaard A, Bach-Mortensen N, Schmidt A. The effect of sodium cromoglycate delivered by Spinhaler and by pressurized aerosol on exercise-induced asthma in children. *Clin Allergy* 1982;12:601-5.
- ⁶ Strauss RH, McFadden ER jun, Ingram RH jun, Jaeger JJ. Enhancement of exercise-induced asthma by cold air. *N Engl J Med* 1977;297:743-7.
- ⁷ Strauss RH, McFadden ER jun, Ingram RH jun, Deal EC jun, Jaeger JJ. Influence of heat and humidity on the airway obstruction induced by exercise in asthma. *J Clin Invest* 1978;61:433-40.
- ⁸ Deal EC jun, McFadden ER jun, Ingram RH jun, Strauss RH, Jaeger JJ. Role of respiratory heat exchange in production of exercise-induced asthma. *J Appl Physiol: Respirat Environ Exercise Physiol* 1979;46:467-75.
- ⁹ Deal EC jun, McFadden ER jun, Ingram RH jun, Jaeger JJ. Hyperpnea and heat flux: initial reaction sequence in exercise-induced asthma. *J Appl Physiol: Respirat Environ Exercise Physiol* 1979;46:476-83.
- ¹⁰ O'Byrne PM, Ryan G, Morris M, et al. Asthma induced by cold air and its relation to nonspecific bronchial responsiveness to methacholine. *Am Rev Respir Dis* 1982;125:281-5.
- ¹¹ O'Byrne PM, Morris M, Roberts R, Hargreave FE. Inhibition of the bronchial response to respiratory heat exchange by increasing doses of terbutaline sulphate. *Thorax* 1982;37:913-7.
- ¹² Latimer KM, O'Byrne PM, Morris MM, Roberts R, Hargreave FE. Bronchoconstriction stimulated by airway cooling: better protection with combined inhalation of terbutaline sulphate and cromolyn sodium than with either alone. *Am Rev Respir Dis* 1983;128:440-3.
- ¹³ O'Byrne PM, Thomson NC, Morris M, Roberts RS, Daniel EE, Hargreave FE. The protective effect of inhaled chlorpheniramine and atropine on bronchoconstriction stimulated by airway cooling. *Am Rev Respir Dis* 1983;128:611-7.
- ¹⁴ Ferrus L, Guenard H, Vardon G, Varenne P. Respiratory water loss. *Respir Physiol* 1980;39:367-81.
- ¹⁵ Weast RC. *Handbook of chemistry and physics*. 58th ed. Cleveland, Ohio: Chemical Rubber, 1977:E-41.
- ¹⁶ Kleinbaum DG, Kupper LL. *Applied regression analysis and other multivariable methods*. Massachusetts: North Scituate, Duxbury Press, 1978:289-309.
- ¹⁷ Kleinbaum DG, Kupper LL. *Applied regression analysis and other multivariable methods*. Massachusetts: North Scituate, Duxbury Press, 1978:277-88.
- ¹⁸ Kleinbaum DG, Kupper LL. *Applied regression analysis and other multivariable methods*. Massachusetts: North Scituate, Duxbury Press, 1978:209-15.
- ¹⁹ Winer BJ. *Statistical principles in experimental design*. New York: McGraw-Hill, 1971:244-314.
- ²⁰ Crapo RO, Morris AH, Gardner RM. Reference spirometric values using techniques and equipment that meet ATS recommendations. *Am Rev Respir Dis* 1981;123:659-64.
- ²¹ Breslin FJ, McFadden ER jun, Ingram RH jun. The effects of cromolyn sodium on the airway response to hyperpnea and cold air in asthma. *Am Rev Respir Dis* 1980;122:11-6.
- ²² Fanta CH, McFadden ER jun, Ingram RH jun. Effects of cromolyn sodium on the response to respiratory heat loss in normal subjects. *Am Rev Respir Dis* 1981;123:161-4.
- ²³ Griffin MP, MacDonald N, McFadden ER jun. Short- and long-term effects of cromolyn sodium on the airway reactivity of asthmatics. *J Allergy Clin Immunol* 1983;71:331-8.
- ²⁴ Patel KR, Berkin KE, Kerr JW. Dose-response study of sodium cromoglycate in exercise-induced asthma. *Thorax* 1982;37:663-6.
- ²⁵ Deal EC jun, Wasserman SI, Soter NA, Ingram RH jun, McFadden ER jun. Evaluation of role played by mediators of immediate hypersensitivity in exercise-induced asthma. *J Clin Invest* 1980;65:659-65.
- ²⁶ Ben-Dov I, Bar-Yishay E, Godfrey S. Refractory period after exercise-induced asthma unexplained by respiratory heat loss. *Am Rev Respir Dis* 1982;125:530-4.
- ²⁷ Lee TH, Assoufi BK, Kay AB. The link between exercise, respiratory heat exchange, and the mast cell in bronchial asthma. *Lancet* 1983;i:520-2.