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Inhibition of mast cell PGD_2 release protects against mannitol-induced airway narrowing

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ABSTRACT: **Mannitol inhalation increases urinary excretion of 9α,11β-prostaglandin F_2 (a metabolite of prostaglandin D_2 and marker of mast cell activation) and leukotriene E_4. The present study tested the hypothesis that β_2-adrenoreceptor agonists and disodium cromoglycate (SCG) protect against mannitol-induced bronchoconstriction by inhibition of mast cell mediator release.**

Fourteen asthmatic subjects inhaled mannitol (mean dose 252±213 mg) in order to induce a fall in forced expiratory volume in one second (FEV_1) of ⩾25%. The same dose was given 15 min after inhalation of formoterol fumarate (24 μg), SCG (40 mg) or placebo. Pre- and post-challenge urine samples were analysed by enzyme immunoassay for 9α,11β-prostaglandin F_2 and leukotriene E_4.

The maximum fall in FEV_1 of 32±10% on placebo was reduced by 95% following formoterol and 63% following SCG. Following placebo, there was an increase in median urinary 9α,11β-prostaglandin F_2 concentration from 61 to 92 ng·mmol creatinine^{-1}, but no significant increase in 9α,11β-prostaglandin F_2 concentration in the presence of either formoterol (69 *versus* 67 ng·mmol creatinine^{-1}) or SCG (66 *versus* 60 ng·mmol creatinine^{-1}). The increase in urinary leukotriene E_4 following placebo (from 19 to 31 ng·mmol creatinine^{-1}) was unaffected by the drugs.

These results support the hypothesis that the drug effect on airway response to mannitol is due to inhibition of mast cell prostaglandin D_2 release.

KEYWORDS: **Cromoglycate, formoterol, leukotriene E_4, mannitol, 9α, 11β-prostaglandin F_2**

Inhalation of mannitol causes the airways of asthmatics, but not those of healthy subjects, to narrow [1]. The postulated mechanism whereby mannitol causes bronchoconstriction is mediator release in response to an increase in osmolarity of the airway surface liquid [2, 3]. The same mechanism has been proposed for exercise-induced bronchoconstriction [4, 5].

Pharmacological agents used to prevent exercise-induced bronchoconstriction also prevent the airway response to mannitol [6, 7]. The response to both exercise and mannitol is thought to be dependent upon the presence of inflammatory cells, such as mast cells and eosinophils, in the airways. The airway response to both stimuli can be inhibited by long-term treatment with inhaled corticosteroids [8, 9].

Nedocromil sodium, which inhibits mediator release from mast cells [10], also inhibits the airway response to mannitol [6]. This finding is consistent with the concept that the airway response to mannitol involves release of bronchoconstricting mediators from airway mast cells. Further evidence for the involvement of mast cells is the finding of reduced airway sensitivity to mannitol in the presence of a histamine receptor (H_1) antagonist, fexofenadine [7]. Leukotrienes (LTs) are also implicated in the airway response to mannitol, particularly in sustaining the bronchoconstriction. This has been demonstrated by the rapid recovery to baseline lung function after challenge with mannitol in the presence of the LT receptor antagonist montelukast [7]. The source of LTs in response to mannitol challenge is unknown, and, unlike histamine, a number of different cell types, in addition to mast cells, could contribute to LT formation.

It has recently been reported that, following inhalation of mannitol, there is an increase in the urinary excretion of 9α,11β-prostaglandin (PG) F_2, a metabolite of the mast-cell-derived PGD_2 [3]. This finding is consistent with mast cell activation and suggests that there is potential for the bronchoconstricting PGs to be involved in the airway response to mannitol. In the same study, a

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sustained (90 min) increase in the urinary excretion of LTE_4, the pulmonary end-metabolite of the cysteinyl-LTs, was reported [3].

It is not known whether drugs that inhibit mediator release from mast cells *in vitro* [11–13] also inhibit mediator release in response to mannitol challenge *in vivo*. In the present study, the effect of a long-acting β_2-agonist and disodium cromoglycate (SCG) on the airway response to mannitol and urinary excretion of mediators was investigated in a double-blind placebo-controlled crossover trial.

METHODS

Study design

Subjects attended the laboratory on four occasions, each at approximately the same time of day and separated by $\geqslant$2 days. The first visit served as a control day, on which a mannitol challenge was performed to assess responsiveness and a skin-prick test to assess atopy. A mannitol challenge was performed with progressively increasing doses, as previously described [1], and forced expiratory volume in one second (FEV_1) was used as the index of change in airway calibre (Microlab 3300 spirometer; Micromedical, Chatham, UK). The challenge was completed when a $\geqslant$25% reduction in FEV_1 was documented or when the maximum cumulative dose (635 mg) of mannitol had been administered.

The second, third and fourth visits are summarised in figure 1. Both drugs were chosen because they could be delivered as a dry powder. In order to maintain blinding and achieve similar deposition to that with mannitol, the formoterol, SCG and placebo were all administered using the Inhalator™ (Boehringer Ingelheim, Ingelheim, Germany). This device shows excellent dispersion characteristics, with the percentage of fine particles found to be maintained or improved using the Inhalator™. The fine particle fraction was 29% for formoterol and 37% for SCG, and compared well with the 25 and 11% fractions obtained with the inhalers normally used to deliver formoterol (Aerolizer™; Novartis Australia, North Ryde, Australia) and SCG (Spinhaler™; Sanofi-Aventis, Macquarie Park, Australia).

Each drug was administered with two inhalations containing either formoterol (12 μg·inhalation^{-1}; Novartis, Basle, Switzerland), SCG (20 mg·inhalation^{-1}; Rhône-Poulenc Rorer, Baulkham Hills, Australia) or placebo. A mannitol challenge was performed 15 min after treatment using the dose increment that caused the 25% fall in FEV_1 on the control day. Recovery of FEV_1 to baseline levels following mannitol challenge was spontaneous and measured at 5 and 10 min and then at 10-min intervals until 90 min after the completion of the challenge. Subjects were asked to drink 100 mL water 1 h before each visit. They were given a further 100 mL at hourly intervals during the visit. Urine samples were collected by asking the subject to void into a clean container. Two baseline samples were obtained, the first on arrival at the laboratory and the second an hour later and immediately before administration of the drug and mannitol challenge (fig. 1). Further urine samples were collected 30, 60 and 90 min after the end of the challenge. All urine samples were shipped on dry ice to the Karolinska Institutet in Stockholm (Sweden) and further stored without preservatives at -20°C until analysis.

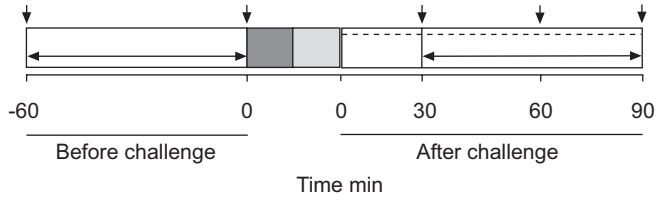

FIGURE 1. Urine was collected at time points (vertical arrows) and over 60-min periods (horizontal arrows) at baseline (-60 min) and before and after mannitol challenge (□) in the presence of formoterol, disodium cromoglycate or placebo. The mannitol challenge, using the cumulative dose that caused a 25% fall in forced expiratory volume in one second (FEV_1) on the control day, commenced 15 min after administration of treatment (■). The time taken to complete the mannitol challenge was 12±3 min (mean±SD; n=14). Recovery of FEV_1 was spontaneous and measured at 10-min intervals for 90 min (------).

Subjects

Nonsmoking atopic asthmatic subjects with a baseline FEV_1 of $\geqslant$70% of the predicted value [14] and without any chest infection in the 4-week period preceding the initial visit (n=35) underwent a control mannitol challenge to assess whether a $\geqslant$25% fall in FEV_1 could be achieved. Fourteen subjects entered the study; the remaining subjects either showed airway responses that were too mild (n=16), did not return for subsequent visits (n=2) or were withdrawn from the study due to either chest infection, viral infection or exacerbation of their asthma (n=3).

Short-acting β_2-agonists were withheld for 6 h and long-acting β_2-agonists, nedocromil and SCG were withheld for 48 h before each study day. Antihistamines were withheld throughout the whole study period, and inhaled corticosteroids on the day of the study. All subjects were required to abstain from caffeine and alcohol from 20:00 h on the evening before the study and no caffeine- or niacin-containing food or drink and no vigorous exercise were permitted on the study day. The Central Sydney Area Health Service Ethics Committee (Camperdown, Australia) approved the study (Protocol No. X02-0171) and all subjects signed a consent form. The study was performed under the Clinical Trials Notification Scheme of the Therapeutic Goods Administration of Australia (Canberra, Australia; CTN# 2002/383).

Mediator analyses

Enzyme immunoassay of 9α,11β-PGF_2 was performed in serially diluted urine samples, using a rabbit polyclonal antiserum and acetylcholinesterase-linked tracer (Cayman Chemical Company, Ann Arbor, MI, USA) essentially as described previously [5]. The antibody cross-reacted with 9α,11β-PGF_2 (100%), 2,3-dinor-9α,11β-PGF_2 (10%), and PGD_2, $PGF_{2\alpha}$ and 8-epi-$PGF_{2\alpha}$ (all <0.01%). Analysis of urinary LTE_4 was performed following a similar protocol [15] employing a rabbit polyclonal antiserum directed against cysteinyl-LTs (Cayman Chemical Company) with acetylcholinesterase-linked LTE_4 as tracer. The specificity of the antiserum for LTC_4 was 100%, for LTD_4 100% and for LTE_4 67%. The detection limit of both assays was 7.8 pg·mL^{-1}. Creatinine analyses were performed using a colorimetric assay (Sigma-Aldrich Sweden, Stockholm, Sweden). All urine samples were

analysed for creatinine and the results expressed as nanograms of excreted mediator per millimole of creatinine.

Statistical analysis

For airway responses, data are expressed as mean±SD. Differences were assessed using either a t-test or ANOVA with repeated measures and relationships were assessed using Pearson's correlation (r_p). Sample size requirements were calculated using the data from a previous study [3]. Results are presented as peak *versus* baseline, with the baseline value expressed as the mean of the values measured at -60 and 0 min and the peak value as the highest value observed at 30, 60 or 90 min after mannitol inhalation. Urinary mediator excretion levels were not normally distributed and are presented as median (interquartile range). Differences between more than two groups were determined using Friedman's repeated-measures analysis of variance on ranks. The difference between two groups was determined using the Wilcoxon signed-rank test. Differences are considered significant if $p<0.05$. The degree of protection (percentage) afforded by formoterol and SCG was calculated by expressing the difference between the maximum percentage fall in FEV1 on the placebo day from the drug treatment days as a percentage of the maximum percentage fall in FEV1 on the placebo day.

RESULTS

Airway response to mannitol

The cumulative dose of mannitol delivered on the control day was 252±213 mg, and this caused a fall in FEV1 of 29±4% (table 1). The same dose of mannitol provoked a 32±10% fall in FEV1 in the presence of placebo in the double-blind session of the study (table 2).

Both SCG and formoterol significantly inhibited the response to inhaled mannitol ($p<0.001$) by providing 63±19 and 95±7% protection of the maximum fall in FEV1, respectively (table 2). No difference in baseline FEV1 on all test days ($p=0.99$) was found; however, after administration of formoterol, there was a 7.6±6.4% increase in FEV1 compared with baseline (fig. 2). This improvement in response to a bronchodilator was small, probably due to the fact that the majority of subjects showed normal FEV1 at baseline (table 1). The protection afforded by formoterol on the airway response to mannitol was virtually complete. The increase in FEV1 due to formoterol was not related to its protective effect on airway sensitivity to mannitol (provocative dose of mannitol causing a 15% fall in FEV1; $r_p=0.07$, $p=0.83$).

Urinary excretion of mediators

In association with the mannitol-induced bronchoconstriction on the placebo day, there was an increase in the urinary excretion of both 9α,11β-PGF_2 and LTE_4. The median (interquartile range) urinary excretion of 9α,11β-PGF_2 and LTE_4 are given at each time point for placebo and both drugs in figure 3. In the presence of placebo, levels of 9α,11β-PGF_2 increased from 61 (45–84) to 92 (63–130) ng·mmol creatinine^{-1} ($p=0.001$) and of LTE_4 from 19 (15–24) to 31 (24–35) ng·mmol creatinine^{-1} ($p<0.001$). Data are expressed as baseline *versus* peak levels after mannitol challenge.

In contrast, there was no significant increase in urinary excretion of 9α,11β-PGF_2 in the presence of either formoterol or SCG. With formoterol, the peak level after mannitol challenge was 67 (48–80) compared with 69 (46–77) ng·mmol creatinine^{-1} at baseline ($p=1.0$), and, in the presence of SCG, the highest post-challenge level of 9α,11β-PGF_2 was 60 (51–96)

TABLE 1 Subject characteristics

Subject No.	Age yrs	Sex	Height cm	Asthma drug	Steroid μg·day^{-1}	Baseline FEV1 % pred	Mannitol		
							Total dose# mg	PD15 mg	Reduction in FEV1¶ %
1	30	M	184	TS, B, FF	400	99.1	475	170.3	34.6
2	30	M	178	S		83.1	635	151.6	25.2
3	32	M	172	S		88.7	155	88.1	31.3
4	30	F	169	S		115.5	155	61.4	34.0
5	25	M	173	S, FP	2000	81.0	635	342.5	31.5
6	20	F	167	TS		94.1	155	85.9	25.7
7	28	F	181	S		69.9	75	15.2	38.7
8	31	M	175	S		79.1	155	88.8	26.1
9	17	F	170	S		115.8	75	40.5	27.2
10	31	M	185	S, FP/SX	500	93.5	475	260.9	25.7
11	19	F	169	S, FP/SX	50	89.2	75	41.1	27.2
12	22	F	173	S, FP	250	93.7	315	174.4	27.2
13	25	F	166	S		84.0	75	42.2	27.9
14	37	F	165	S		85.6	75	30.5	25.3
Mean±SD						91±13	252±213	81 (49–135)+	29±4

FEV1: forced expiratory volume in one second; PD15: provocative dose of mannitol causing a 15% fall in FEV1; M: male; F: female; TS: terbutaline sulphate; B; budesonide; FF: formoterol fumarate; S: salbutamol; FP: fluticasone propionate; SX: salmeterol xinafoate; FP/SX: FP and SX in combination. #: giving rise to ⩾25% fall in FEV1; ¶: maximum on control day; +: geometric mean (95% confidence interval).

TABLE 2 Bronchoprotection by formoterol fumarate and disodium cromoglycate

Subject No.	Control	Placebo	Disodium cromoglycate		Formoterol fumarate	
	Reduction in FEV_1 %	Reduction in FEV_1 %	Reduction in FEV_1 %	Protection %	Reduction in FEV_1 %	Protection %
1	34.6	10.5	4.0	61.5	0.0	100.0
2	25.2	27.8	13.2	52.3	0.0	100.0
3	31.3	18.5	14.3	22.9	0.3	98.3
4	34.0	36.9	16.8	54.4	5.5	85.2
5	31.5	33.6	20.7	38.4	4.4	86.8
6	25.7	33.8	8.8	74.0	4.9	85.4
7	38.7	27.7	5.8	79.1	0.0	100.0
8	26.1	31.6	8.3	73.8	0.0	100.0
9	27.2	38.7	0.0	100.0	0.0	100.0
10	25.7	43.8	16.1	63.3	8.3	81.0
11	27.2	20.4	9.1	55.3	2.1	89.5
12	27.2	47.9	18.9	60.6	0.0	100.0
13	27.9	39.1	17.5	55.3	1.3	96.8
14	25.3	35.8	5.6	84.4	0.0	100.0
Mean±SD	29±4	32±10	11±6	63±19	2±3	95±7

FEV_1: forced expiratory volume in one second.

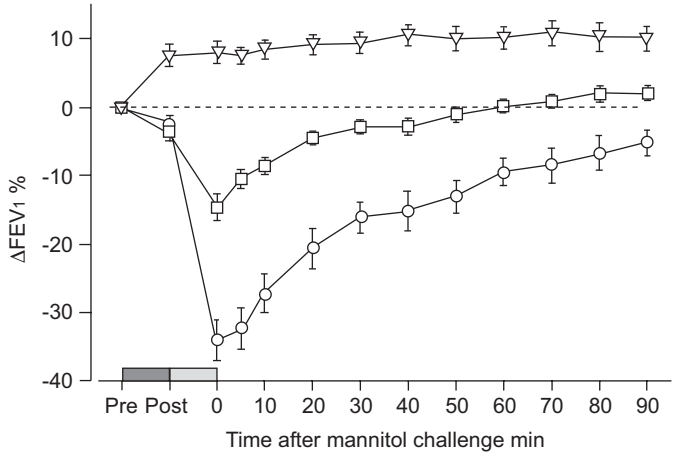

FIGURE 2. Reduction in forced expiratory volume in one second (FEV_1) before (Pre) and 15 min after (Post) inhalation (■) of placebo (○), disodium cromoglycate (□) and formoterol fumarate (▽) and for 90 min after mannitol challenge (■) in 14 asthmatic subjects after a mean±SD cumulative dose of mannitol of 252±213 mg. Data are presented as mean±SD. ·····: baseline values.

compared with 66 (56–85) ng·mmol creatinine^{-1} at baseline (p=0.952). Indeed, the levels of 9α,11β-PGF_2 after mannitol challenge were significantly lower at 90 min compared to baseline in the presence of SCG (p=0.035) and lower at both 60 and 90 min with formoterol (p=0.042 and p=0.049; fig. 3a).

By contrast, the peak *versus* baseline levels of urinary excretion of LTE_4 in the presence of either formoterol or SCG were maintained compared to placebo (fig. 3b). For formoterol, the levels increased from 16 (15–23) to 26 (22–29) ng·mmol creatinine^{-1} (p<0.001), and, in the presence of SCG, from 20 (17–28) to 28 (21–33) ng·mmol creatinine^{-1} (p=0.002).

There were no differences in the baseline levels of urinary 9α,11β-PGF_2 or LTE_4 between the three drug-treated sessions (p=0.931 and p=0.395, respectively).

When urinary excretion over time was expressed as the change in the area under the excretion *versus* time curve (AUC) before and after mannitol challenge, the following results were obtained. Comparing the excretion of 9α,11β-PGF_2 after mannitol challenge between the groups, significantly reduced levels were found after both SCG and formoterol treatment compared to placebo (p=0.013 and p=0.003, respectively). No difference between the two drugs was found (fig. 4a). In the presence of SCG, 9α,11β-PGF_2 excretion was also reduced after mannitol challenge compared to baseline (p=0.02). There was no significant reduction in 9α,11β-PGF_2 excretion in the presence of formoterol compared to baseline (p=0.091; fig. 4a). In contrast, excretion of LTE_4 after mannitol challenge was still increased in the presence of both SCG and formoterol, and the increase in the AUC per hour was not different from that of placebo (p=0.6; fig. 4b).

Five subjects were taking inhaled steroids regularly; these subjects generally tolerated higher doses of mannitol. However, there were no differences in the urinary excretion of mediators between the group taking and that not taking inhaled steroids.

DISCUSSION

This is the first report demonstrating *in vivo* inhibition of the release of a mast cell mediator by SCG and the β_2-agonist formoterol in response to an osmotic stimulus.

It confirms previous findings that inhalation of mannitol is associated with an increase in the urinary excretion of 9α,11β-PGF_2 and LTE_4 [3]. It extends previous findings by demonstrating an inhibitory effect of SCG and formoterol on the excretion of 9α,11β-PGF_2. As urinary levels of methylhistamine

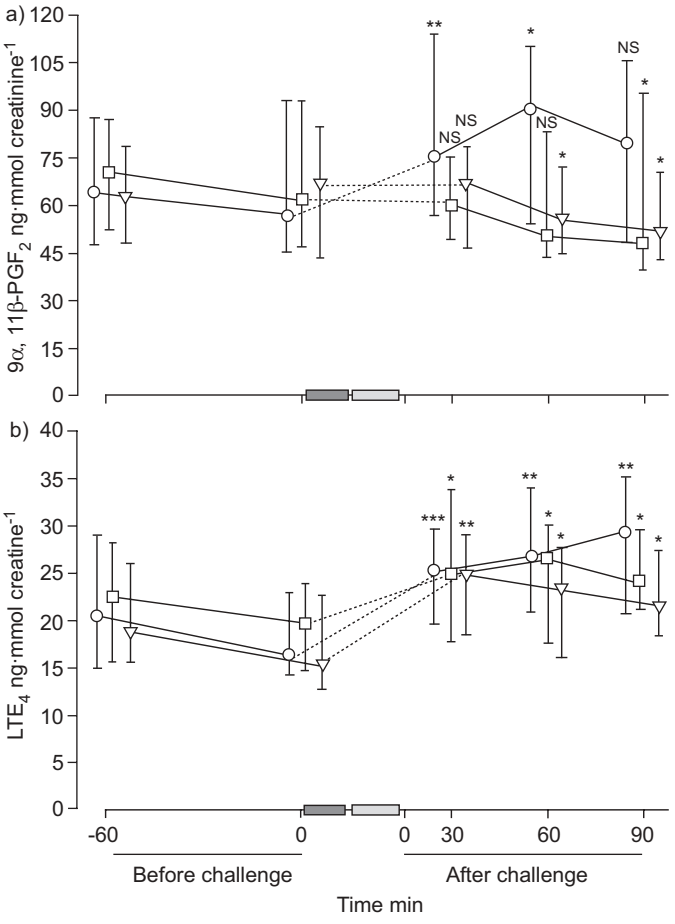

FIGURE 3. Urinary concentration of: a) 9α,11β-prostaglandin (PG) F_2; and b) leukotriene (LT) E_4 over 60 min before and 90 min after mannitol challenge in the presence of placebo (○), disodium cromoglycate (□) and formoterol (▽). Data are presented as median (interquartile range). ·····: period between the two 1-h urine collections, which included the 15 min of treatment (■) and the time taken (mean±SD 12±3 min) to administer the mannitol challenge (■; n=14). NS: nonsignificant. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$ *versus* mean of the two baseline samples.

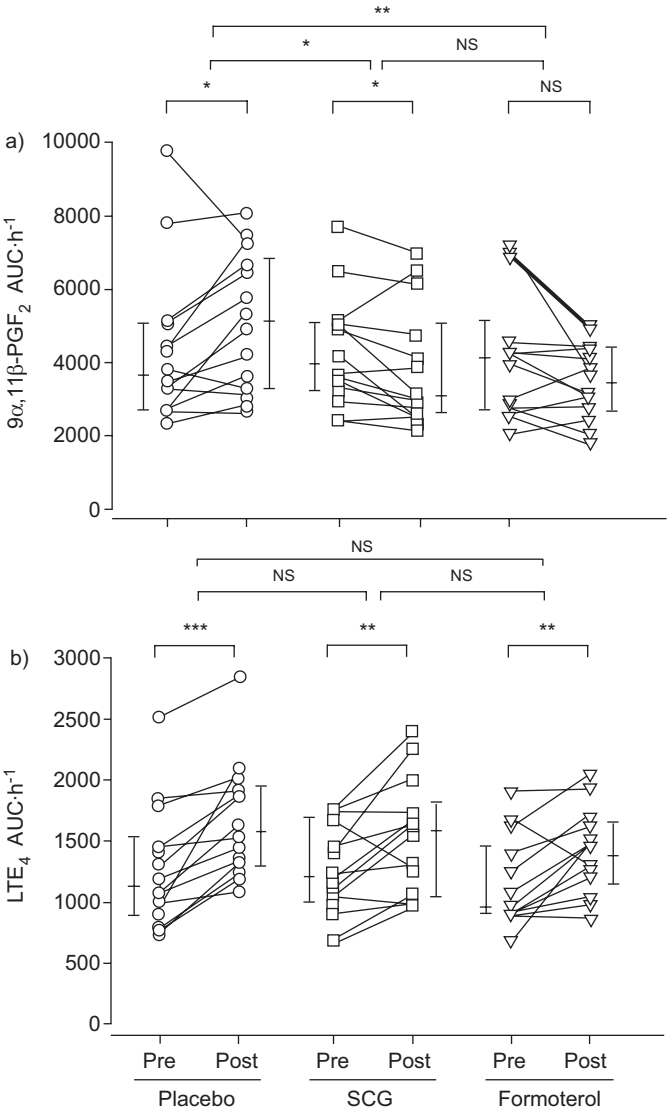

FIGURE 4. Urinary concentration of: a) 9,11β-prostaglandin (PG) F_2; and b) leukotriene (LT) E_4 before (pre) and after (post) mannitol challenge in the presence of placebo, disodium cromoglycate (SCG) or formoterol (n=14). Individual data are shown; vertical bars represent median (interquartile range). AUC: area under the excretion *versus* time curve; NS: nonsignificant. *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$.

were not significantly altered in association with the mannitol-induced bronchoconstriction in the previous study [3], attention was focused upon excretion of the urinary metabolite of PGD_2.

PGD_2 is produced almost exclusively in mast cells and is the predominant cyclooxygenase product in this cell type [16, 17]. Although eosinophils do not possess the capacity to form PGD_2 they can metabolise PGD_2 to 9α,11β-PGF_2 *in vitro* [18]. It is not known to what extent this metabolic route contributed to the urinary level of 9α,11β-PGF_2 found in the present study. Although the number of eosinophils is increased in the airways of asthmatics and eosinophils are a source of cysteinyl-LTs [19], the activity of these drugs on eosinophils in the presence of osmotic stimuli is unknown.

Regarding other potential contributors to PGD_2 release, there are contradictory results concerning macrophages and their capacity to form PGD_2. BALTER *et al*. [20] found PGD_2 in alveolar macrophages derived from bronchoalveolar lavage fluid from asthmatic and control subjects. However, no data are presented regarding the cross-reactivity of the antibody used in their immunoassay or on cell numbers used in experiments. VICENZI *et al*. [21] also found PGD_2 in supernatants from bronchoalveolar macrophages after stimulation with [^{14}C]arachidonic acid. However, again, lack of information regarding the number of cells used makes it difficult to interpret their data.

According to recent data, alveolar macrophages from the bronchoalveolar lavage fluid of healthy subjects stimulated with mannitol do not produce immunoreactive PGD_2 above control levels (unpublished data). This is in contrast to mannitol-stimulated mast cells, which can produce 45-fold increased levels of PGD_2 compared to unstimulated cells [22]. Thus, the possible contribution of PGD_2 released from macrophages in response to hyperosmolar stimulation in the present study seems to be negligible.

The finding of increased concentrations of the PGD_2 metabolite 9α,11β-PGF_2 in urine following challenge with mannitol is in keeping with the previous suggestion that mannitol activates mast cells [3]. Both PGD_2 and 9α,11β-PGF_2 are potent bronchoconstrictors *in vivo*, acting mainly on the TP receptor of bronchial smooth muscle [23]. Subjects suffering from asthma show increased numbers of mast cells in the airway smooth muscle layer compared to normal controls [24] and increased numbers of mast cells in sputum [25]. Also, patients with fatal asthma exhibit a significantly higher number of degranulated mast cells in airway smooth muscle compared with nonasthmatic controls [26].

SCG can prevent histamine and PGD_2 release from immunologically activated human dispersed lung mast cells [10–12], and from mast cells prepared from other tissues [12] or sources [13] *in vitro*. In the present study, the importance of mast cells in osmotically driven bronchoconstriction is further supported by the effect of SCG on the PGD_2 metabolite 9α,11β-PGF_2. The decreased urinary levels of 9α,11β-PGF_2 were also related to protection from bronchoconstriction even though SCG showed no bronchodilatory effect.

Pretreatment with formoterol was more effective and more potent than pretreatment with SCG as regards the airway response following mannitol challenge. It is likely that the superior protection afforded by formoterol is due to the functional antagonism of the bronchoconstricting effects of other mediators on airway smooth muscle. The two different sites of action (*i.e.* mast cell and smooth muscle) of a β_2-agonist have been used to explain the superior protection afforded by an aerosol preparation of a β_2-agonist compared with an oral formulation on airway narrowing in response to exercise [27, 28]. The blocking effect of the β_2-agonist on mast cell release of mediators was thought to be more important in preventing exercise-induced bronchoconstriction than the effects on smooth muscle [29]. There was some airway narrowing in the presence of SCG that could have been due to LTs in the absence of any functional antagonism provided by the formoterol. It is known that SCG is not effective at inhibiting LTD_4-induced bronchoconstriction [30].

Immunological stimulation causes release of both cysteinyl-LTs and PGD_2 from mast cells *in vitro*, whereas hyperosmolar mannitol stimulation causes release of histamine and PGD_2 but only a small amount of LTs [22, 31]. The effects of β_2-agonists on mediator release have previously been investigated in mast cells *in vitro*, and significant inhibition of cysteinyl-LTs, PGD_2 and histamine has been found after immunological stimulation [11, 13].

The current findings lend little support to the concept that mast cells are the source of LTs released in response to mannitol stimulation, and there are other cells, such as eosinophils, that could be the source of LTs produced in the airways. Significant release of LTC_4 from eosinophils has been reported after mannitol stimulation [32]. In the present study, urinary excretion of LTE_4 remained unchanged, a finding that suggests a source of LT other than mast cells. However, LTs are likely to be involved in the airway response to mannitol as the time course of recovery of lung function is faster in the presence of the LT antagonist montelukast [7]. The finding that the release of LTE_4 into the urine is sustained following a mannitol challenge also supports the role of LTs in sustaining airway narrowing to mannitol [3]. LTs are also important in sustaining the airway response to exercise [33]. The cysteinyl-LT antagonist zafirlukast and the histamine antagonist loratadine, given in combination before exercise, significantly inhibited but did not completely block the airway response to exercise [34]. This implicates the importance of other active metabolites, such as cyclooxygenase-derived products. There is some evidence that the cyclooxygenase inhibitor indomethacin, when given by inhalation, provides some protection against exercise-induced bronchoconstriction [35]. However, in a study using a thromboxane receptor antagonist, no protection was afforded against exercise-induced asthma, although the antagonist did inhibit the effects of inhaled PGD_2 in the same subjects [36].

Although the protective effect of formoterol on the airway response to mannitol could be accounted for by functional antagonism of bronchial smooth muscle, this is not the case with SCG. The results, although not definitive of cause and effect, suggest that SCG protected against the airway narrowing induced by mannitol by inhibiting the release of PGD_2. As formoterol also inhibited the increased urinary excretion of the PGD_2 metabolite 9α,11β-PGF_2 following challenge with mannitol, this mode of action could also have contributed to its protective effect. The long-acting β_2-agonist formoterol was chosen because it is available as a powder and exhibits a fast onset of action. The limitation of this study is the measurement of only one specific mast cell mediator PGD_2 and its correlation with airway responses. Future studies may need to include other measurements.

In conclusion, mannitol is known to release mediators from human lung mast cells *in vitro* [31], and specific receptor antagonists have shown that mast cell mediators contribute to the bronchoconstriction caused by mannitol [7]. The present authors consider mast cells located on or near the airway surface to be the most likely source of prostaglandin D_2 since both mannitol and the drugs were delivered as aerosols. The present findings are consistent with the hypothesis that β_2-adrenoreceptor agonists and disodium cromoglycate protect against mannitol-induced bronchoconstriction by inhibition of mast cell mediator release. The authors speculate that this is, at least in part, *via* the inhibition of release of prostaglandin D_2, further implicating the importance of mast cell activation in the airway response to mannitol.

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