

Nasal hyperosmolar challenge with a dry powder of mannitol in patients with allergic rhinitis. Evidence for epithelial cell involvement

H. KOSKELA, M. B. DI SCIASCIO*, S. D. ANDERSON†, M. ANDERSSON‡, H.-K. CHAN§, S. GADALLA¶ and C. KATELARIS**

Department of Respiratory Medicine, Kuopio University Hospital, 70211 Kuopio, Finland, *Institute of Clinical Pathology, University 'G. D'Annunzio', Chieti, Italy, †Department of Respiratory Medicine, Royal Prince Alfred Hospital, Camperdown, NSW Australia 2050, ‡Institute of Respiratory Medicine, Royal Prince Alfred Hospital, Camperdown, NSW Australia 2050, §Department of Pharmacy, University of Sydney, NSW Australia 2006, ¶Department of Renal Medicine, Royal Prince Alfred Hospital, Camperdown, NSW Australia 2050, **Department of Clinical Immunology, The Westmead Centre, Westmead, NSW Australia

Summary

Background The responses to airway hyperosmolar challenges probably involve various inflammatory mediators. However, it is not fully understood which cell type/types are the source of these mediators. Potential cell types include mast cell, epithelial cell and the sensory c-fibre nerve cell.

Objective To clarify which cell types are involved with the mediator response to hyperosmolarity in the human airway.

Methods Ten healthy subjects, 11 patients with nonactive allergic rhinitis, and nine with active allergic rhinitis were challenged intranasally with mannitol powder, and with sham provocation, on separate days. Symptoms were assessed by visual analogue scales and nasal patency by measuring the nasal peak inspiratory flow (nPIF). Nasal lavage fluid levels of α_2 -macroglobulin (an index of plasma extravasation), substance P (an index of sensory nerve cell activation), tryptase (an index of mast cell activation) and 15-hydroxyeicosatetraenoic acid (15-HETE, an index of epithelial cell activation) were analysed.

Results Immediate, although transient burning was the most prominent symptom in all groups whereas only the patients with active rhinitis experienced a fall in nPIF. Mannitol significantly increased the nasal lavage fluid 15-HETE levels in the allergic patients ($P < 0.01$ vs the sham challenge), but not in the healthy subjects. The increase in 15-HETE correlated with nasal symptoms for itching ($r_s = 0.65$, $P = 0.019$) and burning ($r_s = 0.72$, $P = 0.009$). Detectable levels of tryptase was found only in five allergic subjects. Lavage levels of substance P and α_2 -macroglobulin did not change.

Conclusion Epithelial cell seems to be involved with the mediator response to airway hyperosmolar challenge. The roles of sensory c-fibre nerve cell and mast cell remained less clear.

Keywords: rhinitis, allergic, perennial, hay fever, epithelial cells, hypertonic solutions, mannitol, hyperosmolar challenges

Clinical and Experimental Allergy, Vol. 30, pp. 1627–1636. Submitted 8 September 1999; revised 6 January 2000; accepted 2 March 2000.

Introduction

Exercise, hyperventilation with dry/cold air, and inhalation of hypertonic aerosols are known to provoke bronchoconstriction

Correspondence: H. Koskela, Department of Respiratory Medicine, Kuopio University Hospital, 70211 Kuopio, Finland.

in asthmatic subjects. These same stimuli cause nasal obstruction and symptoms in susceptible patients [1–5]. The stimulus for these airway responses is likely to be the increase in the osmolality of the airway lining fluid (ALF) and cells in the airway mucosa [6,7].

The mechanism whereby hyperosmolarity leads to bronchoconstriction in asthmatics and symptoms in certain patients with rhinitis is not precisely known but is thought to involve the release of inflammatory mediators. Such mediators include leukotrienes, prostaglandins and histamine [8]. Evidence in support of mediator release comes from studies showing the inhibitory effects of specific antihistamines, leukotriene antagonists and prostaglandin (synthetase) inhibitors such as flurbiprofen on challenges with hyperpnea and hypertonic saline in asthmatic subjects [8,9]. In the nose the involvement of these same mediators has been suggested although their role is clearer for challenge with dry air [2,10] than hyperosmolarity [4,10,11].

These mediators can be produced by a variety of cell types. It is not fully understood which cell type(s) are involved with hyperosmolar challenges although mast cells and epithelial cells have been proposed [12]. Another potential source of mediators is the sensory c-fibre nerve cell which can be stimulated by hyperosmolarity and can release local mediators such as substance P and neurokinin A [13–16]. A better understanding about the roles of various cell types in the responses to hyperosmolar challenges might have therapeutic implications.

The primary purpose of this study was to clarify which cell types are involved with the mediator response to hyperosmolarity in the human airway. We focused on the airway epithelial cell, mast cell, and sensory c-fibre nerve cell, the three most likely cell 'candidates' [8]. We studied the effects of hyperosmolar challenge on the nasal airway in patients with active and nonactive allergic rhinitis as well as in healthy subjects. The nose was chosen as a challenge site for the following reasons: first, collecting specimens for the analysis of mediators can be easily made from the nose. Second, the nose can be challenged without fear of life-threatening responses. The latter feature allows the use of a single, large dose challenge. Third, encouraged by our experience in lower airways [17,18], we wanted to test whether a nasal challenge with mannitol might also be clinically useful for assessing patients with allergic rhinitis.

Materials and methods

Subjects

There were 20 patients with allergic rhinitis. They all showed at least one positive ($>3 \times 3$ mm weal) reaction to common aeroallergens (dust mite, grass, fungi, animal dander) on skin testing (Allergenic Extract, Miles Inc.,

Elkhart, IN, USA). We intended to recruit both patients with currently active symptoms and patients with nonactive symptoms. Prior to the challenges the patients filled in a questionnaire in which they were asked whether their rhinitis is worse at a particular time of year. The patients who answered yes to this question and whose symptomatic period was outside the study period (beginning of autumn) formed the group 'nonactive rhinitis' ($n=11$). The group 'active rhinitis' ($n=9$) comprised eight subjects who had perennial symptoms of rhinitis plus one subject who had seasonal variation in her disease but who had her symptomatic period at the time the study was carried out. Ten healthy control subjects were also recruited. They had no history of asthma or rhinitis, suffered from no chronic disease, took no regular medication, and had no positive skin reactions to common aeroallergens and were nonsmokers. None of the 30 subjects had had a respiratory tract infection within 6 weeks before the study. Table 1 shows the basic characteristics of the subjects.

Of the patients with nonactive rhinitis, none used regular medications. Of the patients with active rhinitis, two used intranasal corticosteroids, four used oral antihistamines and one intranasal decongestants occasionally. The patients were asked to withhold their intranasal steroids for 2 weeks, astemizole for 2 weeks, cetirizine for 1 week, other oral antihistamines for 48 h, and intranasal decongestants for 24 h prior to the challenges.

All subjects gave their informed written consent for participation in the study, which was approved by the local ethics committee, protocol number X97–0273. The mannitol was used under the Clinical Trials Notification Scheme of the Therapeutic Goods Administrations of Australia No. CTN 1997/185.

Study design

The study was performed on 2 separate days. On the first day the subjects were interviewed, the skin prick tests were performed (if not done within 1 year of the study), and the patient familiarized with the visual analogue scales (VAS). In addition, the nasal inspiratory manoeuvres were practised several times. Then the subjects were challenged, either with a capsule containing dry powder of mannitol, or with an empty capsule (the sham challenge), in single blind fashion. Within 11 days they returned to perform the other challenge, in random order. All but two subjects had at least 1 day between the challenges. Spirometry was performed before and after the challenges as a safety measure.

The challenge

Firstly, symptoms were assessed using the VAS and then the baseline nasal lavage was performed. Immediately after that

Table 1. Basic characteristics of the subjects

	Healthy	Non-active rhinitis	Active rhinitis
Number of subjects	10	11	9
Male/female	5/5	5/6	3/6
Age (years)	34 ± 10	34 ± 14	28 ± 6
Skin reaction to <i>D. pteronyssinus</i> (mm)		5.0 ± 2.4	7.4 ± 3.1*
Skin reaction to ryegrass (mm)		9.1 ± 10.9	3.0 ± 3.6†
Duration of rhinitis (years)		19 ± 18	12 ± 8

Figures are means and standard deviations. * $P = 0.04$ and † $P = 0.07$ between the groups 'nonactive rhinitis' and 'active rhinitis'.

peak nasal inspiratory flow (nPIF) was measured in triplicate using the Youtlen nPIF meter (Clement Clarke International Ltd, Essex, England) and the highest value was used for analysis. After that, three forced expiratory manoeuvres were performed through the mouth (Autospiro AS-300, Minato Medical Science Co., Osaka, Japan), and the best FEV₁ was used for analysis. After the baseline lavage, the subjects rested 30 min while breathing through the nose in order to evaporate the excessive fluid from the nose before the powder application. The contents of the capsules were applied to nostrils using Teijin (Teijin Ltd, Tokyo, Japan) insufflators, separate capsules and devices for each nostril. Before the application the subjects were asked to take a deep breath and then hold their breath during the application. The device was inserted to the left nostril and actuated 20 times after which the investigator pinched the subject's nose and asked the subject to exhale through the mouth. After that the right nostril was challenged. Before and after the applications, the devices plus the capsules were weighed. If less than 25 mg of mannitol had escaped from each capsule, the actuations were repeated within 10 seconds. The sham challenge was identical but an empty capsule was used. Sneezes were counted from the application of the powder up to the third (the last) lavage.

Thirty seconds after the end of the application of the powder or sham, the subject was asked to assess her/his symptoms. At two minutes nasal lavage was performed and after that, the nPIF was measured in triplicate. At 10 minutes the symptom assessment, lavage and nPIF were repeated. Finally, the FEV₁ was measured in triplicate.

The mannitol powder

The preparation of mannitol powder has been described in detail previously [17]. However, this time we spray dried a solution containing 200 mg mannitol/mL whereas in the previous study we used a solution of 15 mg/mL. A higher concentration was used to achieve larger particles to ensure

upper airway deposition of the powder. The particle size of the powdered mannitol was measured (Mastersizer S Version 2.15, Malvern Instruments Ltd, Malvern, UK) and the percentage of the particle mass in the mannitol aerosol cloud over 6.6 µm was 72%. The batch of mannitol powder was irradiated with approximately 4.7 kGy at Steritech (Wetherill Park, Australia). Gelatin capsules were hand-filled with 50 ± 5 mg of mannitol powder.

VAS lines

Five 120 mm horizontal lines were drawn on a page and labelled 'nasal running, nasal blocking, nasal itching, nasal burning, and itching of the throat', respectively. Under the left end of each line there was a text 'not at all' and under the right end of each line 'extremely severe'. The subjects made a vertical marking on each line with a sharp pencil to describe their present symptom severity. The distance of that marking from the left end of the line was measured to a resolution of 1 mm.

Nasal lavage and handling of the samples

Our method was a modification of that described by Naclerio *et al.* [19]. The subjects extended their necks approximately 45° from the vertical while in a sitting position. They were asked to take a deep breath and then hold their breath. Five millilitres of normal saline (0.9%) were instilled into each nostril. Then, the subjects were asked to pinch their nose and bend their necks forward approximately 60° from the vertical. The subjects could now start breathing through the mouth. This way the fluid could be kept in the nose for 60 s until the subjects opened their nose and let the fluid flow through a funnel into a tube. After that, the subjects gently blew their nostrils into the funnel.

Immediately after collection of the sample, protease inhibitors were added. The amounts were calculated depending on the volume of the returning fluid to

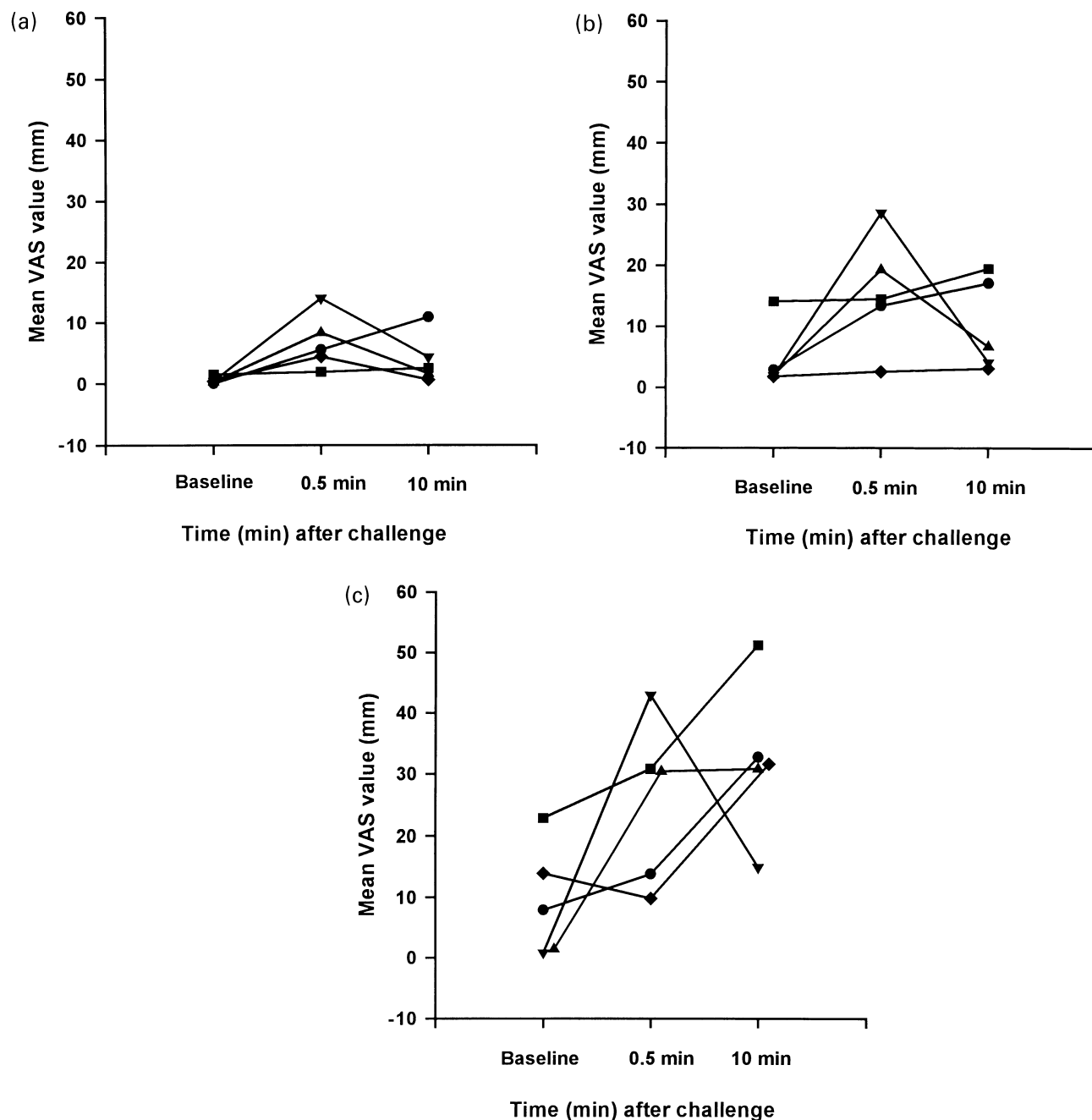


Fig. 1. Nasal symptoms. Each point represents the mean of the patient groups' markings on visual analogy scale (VAS) during the mannitol challenge. Error bars are omitted for clarity. (a) Healthy subjects ($n = 10$); (b) Patients with nonactive allergic rhinitis ($n = 11$); (c) Patients with active allergic rhinitis ($n = 9$). —●— Running; —■— Blocking; —▲— Itching; —▼— Burning; —◆— Throat itching.

achieve final concentrations of 2×10^{-5} M captopril (ICN Biomedicals Inc., Aurora, Ohio, USA) and 2×10^{-5} M phosphoramidon (ICN Biomedicals Inc., Aurora, Ohio, USA). The tubes were shaken and then immersed in ice until the end of the challenge. After that the tubes were centrifuged at 4°C , $1000g$ for 20 min and the supernatants were frozen (-80°C) and stored for later analysis.

Analytical assays of the nasal lavage fluid

15-Hydroxyeicosatetraenoic acid (15-HETE), an index of epithelial cell activation [20–22], was measured with a commercially available radioimmunoassay (RIA) kit according to the manufacturer's instructions (15-HETE ^3H RIA Kit, PerSeptive Biosystems, Inc., Framingham, MA,

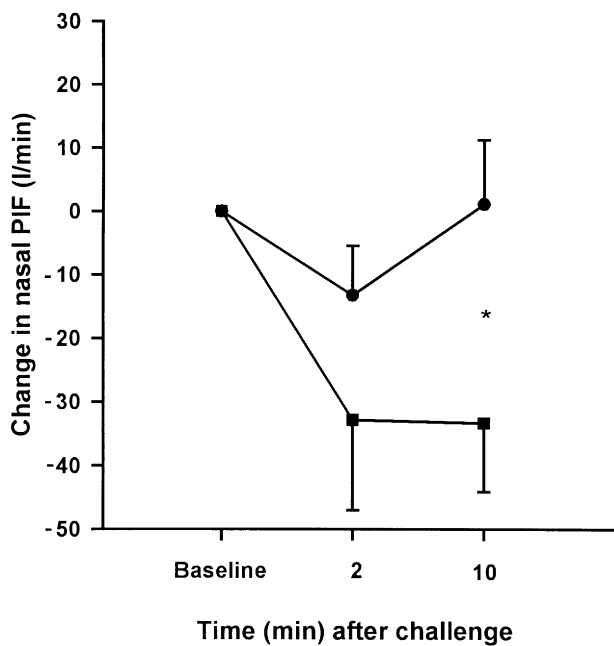


Fig. 2. Changes in nasal peak inspiratory flow (nPIF) values induced by the challenges in patients with active allergic rhinitis ($n=9$). * $P<0.05$. —●— Sham challenge; —■— Mannitol challenge.

USA). Owing to the lack of further availability of test kits from the company, 15-HETE concentrations could be analysed in 20 subjects only. Six subjects were randomly chosen from the healthy group and 14 from the entire group of 20 allergic patients. The sensitivity of the RIA was $2.5 \text{ pg}/0.1 \text{ mL}$. Tryptase, an index of mast cell activation [23] was measured with a commercially available fluoroenzymeimmunoassay kit (UniCAP Tryptase Fluoroenzymeimmunoassay, Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden), with a sensitivity of $1 \mu\text{g}/\text{L}$. Substance P, an index of sensory c-fibre nerve cell activation [24] was also measured with a commercially available RIA kit according to manufacturer's instructions (Substance P RIA, Peninsula Laboratories, Inc., Belmont, CA, USA). To investigate whether our challenges induced plasma extravasation, α_2 -macroglobulin was measured with RIA as described by Svensson *et al.* with a sensitivity of $0.1 \text{ mg}/\text{L}$ [25].

Statistical analysis

Non-parametric tests were used, the Wilcoxon signed rank test for paired and Mann-Whitney *U*-test for unpaired comparisons. The comparisons between the three groups were done using Kruskal-Wallis test. Spearman rank correlation test (r_s) was used for calculations of correlation. All values are given as means $\pm$ SEM and a *P*-value less than 0.05 was considered significant.

Results

The total amounts of mannitol delivered to the subjects' noses were 64.7 ± 2.5 , 70.1 ± 3.7 and $64.7 \pm 2.9 \text{ mg}$ in the groups 'healthy', 'nonactive rhinitis' and 'active rhinitis', respectively ($P=0.4$).

Symptoms

Not surprisingly, there were clear differences in baseline symptoms between the groups. The scores at baseline for all the five symptoms for the 2 test days were added and the sum divided by two. There were statistically highly significant differences between these values (4.0 ± 1.7 , 28.3 ± 12.2 and $71.8 \pm 18.7 \text{ mm}$ in the groups 'healthy', 'nonactive rhinitis', and 'active rhinitis', respectively ($P=0.0005$). As the baseline symptoms greatly differed between the groups, the mannitol-induced changes in symptoms were not statistically compared between the groups.

Thirty seconds after the challenge burning was the most intense sensation within all groups (Fig. 1a,b,c) and was also the symptom which appeared first most commonly (20 out of 30 subjects). Figure 1(c), illustrating the symptoms in the 'active rhinitis' group, shows most clearly that burning and itching were the early symptoms with running, blocking and itchy throat being most intense 10 min after challenge.

Sneezes

Sneezes were infrequent. After the sham challenge one patient sneezed and four sneezed after the mannitol challenge. There were 1–7 sneezes per patient.

The nPIF values

There was no statistically significant difference in the baseline nPIF values between the study days and the mean baseline nPIF values did not differ significantly between the three groups. Mannitol had no effect on nPIF values in the healthy subjects and in the patients with nonactive rhinitis (data not shown) but it decreased nPIF significantly in the group 'active rhinitis' (Fig. 2).

Spirometry

Neither the sham challenge nor the mannitol challenge changed the FEV_1 values ($P=0.66$ and $P=0.71$, respectively, $n=30$). This was true also within each of the three subgroups.

15-HETE

Owing to rather small numbers of patients in the analysis of

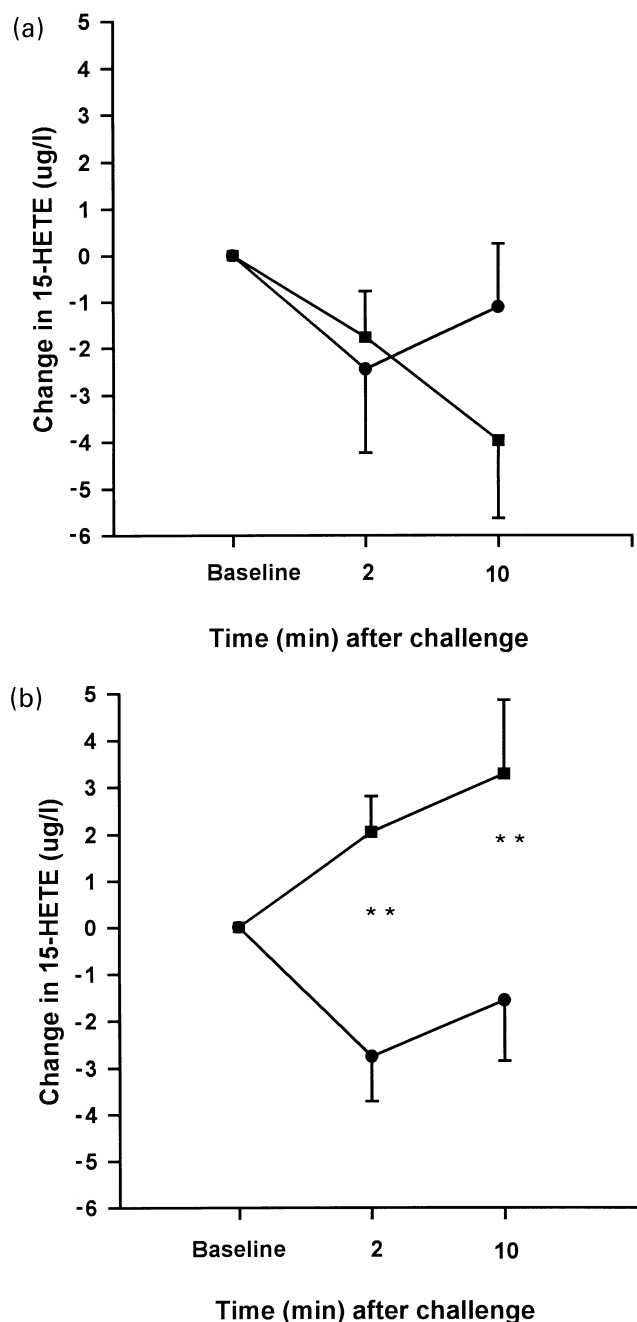


Fig. 3. Changes in nasal lavage fluid 15-hydroxyeicosatetraenoic acid (15-HETE), concentrations induced by the challenges. (a) Healthy subjects ($n = 6$); (b) Allergic patients ($n = 14$). ** $P < 0.01$. —●— Sham challenge; —■— Mannitol challenge.

15-HETE, the groups 'nonactive rhinitis' ($n = 6$) and 'active rhinitis' ($n = 8$) were combined as an 'allergic' group ($n = 14$). Lavage levels from six healthy subjects were also analysed. The baseline 15-HETE concentration did not differ significantly between the two study days ($P = 0.69$) and the mean baseline 15-HETE concentrations

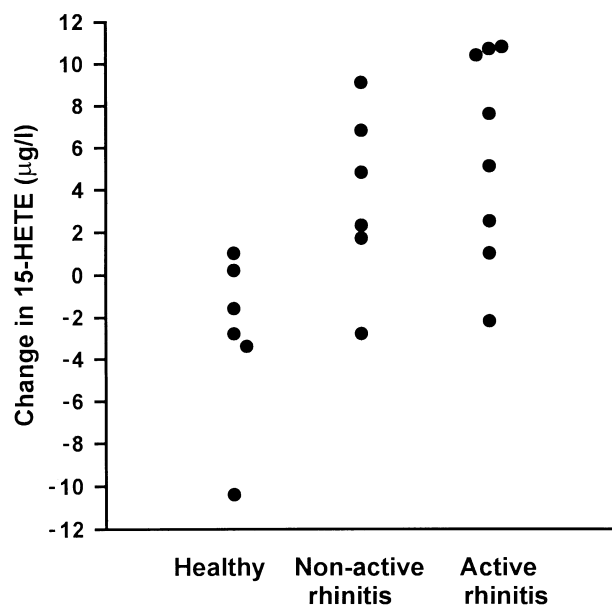


Fig. 4. Changes in nasal lavage fluid 15-hydroxyeicosatetraenoic acid (15-HETE), concentrations in each subject at 10 min after the challenges. The values are calculated as the change from baseline induced by the mannitol challenge minus the change induced by the sham challenge.

did not differ between the healthy and allergic subjects (8.8 ± 2.1 and 10.4 ± 1.1 (g/L, respectively, $P = 0.41$).

In healthy subjects, mannitol challenge, compared with the sham challenge, did not change the levels of 15-HETE significantly at either time points after the challenge (Fig. 3a). Actually, mannitol challenge tended to decrease the 15-HETE levels in the healthy subjects. By contrast, for the 14 allergic subjects mannitol significantly increased the 15-HETE concentrations, compared with the sham challenge, both at 2 and 10 min after the challenge ($P = 0.002$ and $P = 0.006$, respectively, Fig. 3b). The changes in 15-HETE in each subject are expressed in Fig. 4.

In the allergic subjects, the mannitol-induced increase in 15-HETE concentration at 2 min after the challenge was significantly related to the mannitol-induced increases in itching ($n = 14$, $r_s = 0.65$, $P = 0.019$) and burning ($n = 14$, $r_s = 0.72$, $P = 0.009$) at 10 min after the challenge.

Tryptase

Detectable levels of tryptase at one or more time points could only be found in five subjects. All of them were allergic with three from the 'nonactive rhinitis' group and two from the 'active rhinitis' group. In all these subjects, the highest concentrations of tryptase was found 10 min after the mannitol challenge. At that time, the concentration of tryptase for these subjects was significantly higher after

Table 2. Nasal lavage fluid levels of substance P and α -2 macroglobulin during the sham and mannitol challenges

Substance	Group	Sham baseline	Sham at 2 min	Sham at 10 min	Mannitol baseline	Mannitol at 2 min	Mannitol at 10 min
Substance P ng/l (mean $\pm$ SEM)	Healthy	4.8 $\pm$ 0.9	2.5 $\pm$ 0.6	1.9 $\pm$ 0.6	5.1 $\pm$ 0.8	3.4 $\pm$ 0.6	2.2 $\pm$ 0.5
	Nonactive rhinitis	7.0 $\pm$ 1.3	5.8 $\pm$ 1.6	3.3 $\pm$ 0.8	7.8 $\pm$ 1.8	4.8 $\pm$ 0.8	5.5 $\pm$ 1.9
	Active rhinitis	8.6 $\pm$ 2.5	6.2 $\pm$ 2.1	3.8 $\pm$ 1.7	6.5 $\pm$ 1.7	3.8 $\pm$ 1.1	2.4 $\pm$ 0.7
α -2 macroglobulin mg/l (mean $\pm$ SEM)	Healthy	0.5 $\pm$ 0.2	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.5 $\pm$ 0.2	0.5 $\pm$ 0.3	0.2 $\pm$ 0.1
	Nonactive rhinitis	0.5 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.5 $\pm$ 0.2	0.4 $\pm$ 0.1	0.4 $\pm$ 0.2
	Active rhinitis	0.5 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.4 $\pm$ 0.1

mannitol challenge than after sham challenge (6.1 ± 3.3 vs 0.5 ± 0.5 (grams/l, $P = 0.043$, $n = 5$).

The occurrence of a release of tryptase was not associated with more severe symptoms. The total symptom score did not differ significantly between those subjects with a tryptase release and those without (247.2 ± 101.1 and 101.6 ± 25.1 , $n = 30$, $P = 0.19$).

Substance P

There were no significant difference in the baseline substance P levels between the study days and between the three groups. The changes in substance P concentrations from baseline values did not differ between the mannitol challenge and the sham challenge at any time point for any of the groups. Actually, the levels seemed to decrease in response to the concomitant lavages. (Table 2).

α_2 -macroglobulin

There were no significant differences in the baseline α_2 -macroglobulin levels between the study days and between the three groups. The changes in α_2 -macroglobulin concentrations from baseline values did not differ between the mannitol challenge and the sham challenge at any time point for any of the groups (Table 2). Again, the levels seemed to decrease in response to the concomitant lavages.

Discussion

This present study describes a new nasal challenge that uses a dry powder of mannitol. The results of the present study show that mannitol powder, applied to nasal mucosa, causes immediate symptoms, especially burning of the nose, both in healthy subjects and in subjects with allergic rhinitis. However, only the subjects with active rhinitis had a decrease in nPIF. Sneezing occurred infrequently after nasal challenge with mannitol powder. The results from the mediator assays suggest that the the airway epithelial

cell is involved with the response to hyperosmolar stimulus. This was demonstrated by the significant increase in 15-HETE in the nasal lavage fluid in the allergic subjects, whereas a tendency to decreased 15-HETE levels after mannitol was observed in the healthy subjects

The findings on symptoms agree with previous studies showing that the most prominent immediate symptom to a sudden increase in nasal mucosa osmolarity is burning [3] or a 'tingling' [4] sensation. Though the comparison of the symptomatic response was difficult between the groups, due to the large differences in baseline symptoms, it seemed that the response was greatest in the subjects with active rhinitis. In these patients, nasal blocking by mannitol could also be demonstrated objectively by the decrease in nPIF. This probable association of the symptomatic response with the state of mucosal inflammation makes nasal challenge with mannitol a potential clinical test for monitoring the state of allergic inflammation in the nose. For example, it could be used to assess the response to anti-inflammatory therapy. However, further work with respect to the repeatability of the challenge and dose-response measurements need to be carried out before a clinical application of this test could be recommended.

The significant rise in 15-HETE after mannitol challenge in the allergic subjects strongly suggests activation of the nasal airway epithelial cell. 15-HETE is the major arachidonic acid metabolite released from human nasal, tracheal, and bronchial epithelial cells [20–22]. Of other cell types in the airways, only the eosinophil granulocyte can produce it in significant amounts, though 10 times less than epithelial cell under identical conditions [20–26]. It has been shown previously that dispersed nasal mucosal cells of subjects with nonallergic rhinitis release 15-HETE in response to a rise in the osmolarity of the culture medium [27]. To our knowledge, our study is the first to demonstrate an increase in 15-HETE by hyperosmolarity *in vivo*. As the increase in 15-HETE concentration significantly correlated with the increases in itching and burning of the nose, we speculate that the symptomatic response to

nasal hyperosmolar challenge may be, at least partly, due to a release of inflammatory mediators produced by the nasal airway epithelial cell. However, the most important mediator/mediators responsible for the symptoms and other consequences of hyperosmolar challenges may well be some other than 15-HETE. We stress that in our study 15-HETE only served as an indicator of epithelial cell activation.

This study highlights the role of epithelial cell in the nasal airway response to hyperosmolarity. This is not surprising, as of the various cell types in the airways, the epithelial cell is probably most numerous and is situated most closely to airway lining fluid, in which the largest change in osmolarity probably takes place. Our results support the view of Anderson *et al.* who have come to similar conclusion when analysing the protective effect of various drugs, especially cromones, on responses to hyperosmolar stimuli [12]. The airway epithelial cell is special among the epithelial cells in that the water permeabilities of the apical and basolateral cell membranes are not equivalent. This feature makes the airway epithelial cell an 'osmotic sensor' in the airways. Hyperosmolarity shrinks the epithelial cell, which may initialize various cellular responses [28].

Detectable levels of tryptase could only be found in five allergic patients. Tryptase is a preformed mediator and the major neutral protease of the human mast cell [23]. Though all of the five patients showed the highest concentrations 10 min after mannitol challenge, it has to be noted that we do not know how the tryptase levels behaved in those 25 subjects in whom the tryptase could not be assessed by our methods. Furthermore, previous investigators have usually failed to show a mast cell activation after a hyperosmolar challenge, especially when using tryptase as a mast cell indicator [10,30]. In conclusion, our study could not clarify whether hyperosmolarity can induce mast cell to release preformed mediators, like tryptase, *in vivo*.

Our failure to demonstrate an increase in substance P by mannitol does not necessarily exclude involvement of sensory nerves in the hyperosmolarity-induced responses. It has been shown in animals that c-type nerve fibres, which contain substance P, are irritated by hyperosmolarity [14,15]. In humans, the secretory response to a nasal hyperosmolar challenge is most likely a central reflex. In this case capsaicin-sensitive c-fibre sensory nerves serve as the afferent arch [31,32]. In addition, pretreatment with capsaicin almost abolishes the painful sensation caused by nasal application of hypertonic saline [33]. In this regard our findings also suggest an involvement of sensory nerves because the most consistent symptom after mannitol challenge was the almost immediate burning. In conclusion, we suggest that the role of the substance P-containing (capsaicin-sensitive) sensory nerves in the responses to local hyperosmolarity in the nose is predominantly passive (as an afferent arch of a central reflex and pain sensation) and

not active (as a source of locally acting inflammatory mediators). This view is in keeping with that of Baraniuk who states that: 'The most important effects of sensory nerve stimulation in the nose are the recruitment of central reflexes, and the induction of the sensation of pain' [24].

We did not find an increase α_2 -macroglobulin concentrations in the nasal lavage fluid when compared with the sham challenge. This supports the findings of other investigators that hyperosmolarity does not induce plasma extravasation in human nasal respiratory mucosa [4–10,34]. As nasal sensory nerves probably were involved in our mannitol challenge, our findings support the view that human airways respond in a different way to neurogenic stimuli than rodent airways [35,36]. Neurogenic exudative inflammation seems to be a typical response pattern of rodents but may not exist in human airways.

The failure to detect an increase in α_2 -macroglobulin is also of interest when interpreting the rise in 15-HETE concentration after mannitol challenge. We think that this increase in 15-HETE reflects a true epithelial cell activation and not simply a 'flushing' of the epithelium by extravasated plasma.

We performed a single baseline lavage 30 min before challenge rather than perform several baseline lavages as suggested previously [19]. The rationale for several baseline lavages is to decrease the levels of mediators before challenge in order to detect the changes in mediator concentrations induced by the challenge more efficiently. It is well known that there is a significant concentration of histamine in the nose at rest, even in healthy subjects [37]. However, we were interested in other substances, such as substance P and tryptase, the concentrations of which are known to be low in nasal lavage fluid [10,38]. Therefore, we thought that several baseline lavages could have decreased the concentrations of these substances below the limits of detection of the present assay methods making changes difficult to measure. The potential for low concentrations of mediators in the nasal lavage fluid was also the reason why we kept the lavage fluid in the nose for 60 s instead of the 10 s used before [19]. We assumed that keeping the lavage fluid in the nose for longer could increase the concentrations of mediators. A longer than 10 s lavage was also used by Greiff *et al.* but their method for nasal lavage is quite different from ours [39].

A sham challenge is essential in this kind of study as it helps to identify the dilution effect of repeated lavages on various substances. A challenge with empty capsules was considered a reasonable sham as during the application of mannitol powder by the insufflator air was blown to the nose, a stimulus which by itself could cause symptoms and release of mediators [2]. We used empty capsules in our sham challenge because any powder would have the potential to alter osmolarity. While we considered the use of

lactose as a sham powder initially in our pilot studies, it was found that nasal challenge with lactose also produced the typical burning sensation of the nose.

In conclusion, we have introduced a new nasal challenge method using a dry powder preparation of mannitol and used it as a model to describe human airway response to a local rise in osmolarity. In allergic patients mannitol-induced symptoms are associated with a release of 15-HETE, an epithelial cell derived inflammatory mediator. Our results suggest that the airway epithelial cell is involved with the response to airway hyperosmolar challenge in man. Thus, medical therapy against hyperosmolar challenges, including exercise, should take the epithelial cell into account.

Acknowledgements

This study was funded by the National Health and Medical Research Council of Australia, Finnish Academy, Finnish Anti-Tuberculosis Association, Kuopio University Hospital, Finnish Medical Foundation, Swedish Medical Research Council, Vårdal Foundation, Swedish Otorhinolaryngology Society, Royal Fysiografic Society, and Astra, Sweden. Teijin nasal insufflators were kindly provided by Teijin Ltd, Tokyo, Japan.

We would like to thank Therese Burke, Fiona Carrozzi, Dana Bird, Victoria Lazzaro and Stratos Fotinos for technical assistance.

The application for mannitol described in this paper is covered by US Patent no. 5817 028 and internationally PCT AU95/00086 and is owned by the Central Sydney Area Health Service.

References

- 1 Togias AG, Naclerio RM, Proud D, et al. Nasal challenge with cold, dry air results in release of inflammatory mediators. *J Clin Invest* 1985; 76:1375–81.
- 2 Togias AG, Naclerio RM, Peters SP et al. Local generation of sulfidopeptide leukotrienes upon nasal provocation with cold, dry air. *Am Rev Respir Dis* 1986; 133:1133–7.
- 3 Krayenbuhl MC, Hudspeth BN, Scadding GK, Brostoff J. Nasal response to allergen and hyperosmolar saline. *Clin Allergy* 1988; 18:157–64.
- 4 Silber G, Proud D, Warner J, et al. *In vivo* release of inflammatory mediators by hyperosmolar solutions. *Am Rev Respir Dis* 1988; 137:606–12.
- 5 Anderson SD, Togias AG. Dry air and hyperosmolar challenge in asthma and rhinitis. In: Busse WW, Holgate ST. eds. *Asthma and Rhinitis* 1st edn. Boston: Blackwell Scientific Publications, 1995:1178–95.
- 6 Anderson SD. Is there a unifying hypothesis for exercise-induced asthma? *J Allergy Clin Immunol* 1984; 73:660–5.
- 7 Anderson SD, Smith CM. Osmotic challenges in the assessment of bronchial hyperresponsiveness. *Am Rev Respir Dis* 1991; 143:S43–S46.
- 8 Anderson SD. Inflammatory mediators and exercise-induced asthma. In: Holgate ST, Busse WW. eds. *Inflammatory Mechanisms in Asthma* 1st edn. New York: Marcel Dekker, Inc., 1998: 861–90.
- 9 Finnerty JP, Holgate ST. Evidence for the roles of histamine and prostaglandins as mediators in exercise-induced asthma: the inhibitory effect of terfenadine and flurbiprofen alone and in combination. *Eur Respir J* 1990; 3:540–7.
- 10 Proud D, Bailey GS, Naclerio RM, et al. Tryptase and histamine as markers to evaluate mast cell activation during the responses to nasal challenge with allergen, cold, dry air, and hyperosmolar solutions. *J Allergy Clin Immunol* 1992; 89:1098–110.
- 11 Krayenbuhl MC, Hudspeth BN, Brostoff J, Scadding GK, Guesdon JL, Latchman Y. Nasal histamine release following hyperosmolar challenge. *Allergy* 1989; 44:25–9.
- 12 Anderson SD, Rodwell LT, Daviskas E, Spring JF, du Toit J. The protective effect of nedocromil sodium and other drugs on airway narrowing provoked by hyperosmolar stimuli: a role for the airway epithelium? *J Allergy Clin Immunol* 1996; 98: (No 5 Part; 2):S124–34.
- 13 Jongejan RC, de Jongste JC, Raatgeep RC, Stijnen T, Bonta IL, Kerrebijn KF. Effect of hyperosmolarity on human isolated central airways. *Br J Pharmacol* 1991; 102:931–7.
- 14 Pisarri TE, Jonzon A, Coleridge HM, Coleridge JCG. Vagal afferent and reflex responses to changes in surface osmolarity in lower airways of dogs. *J Appl Physiol* 1992; 73:2305–13.
- 15 Fox AJ, Barnes PJ, Dray A. Stimulation of guinea-pig tracheal afferent fibres by non-isosmotic and low-chloride stimuli and the effect of frusemide. *J Physiol* 1995; 482:179–87.
- 16 Garland A, Jordan JE, Necheles J, et al. Hypertonicity, but not hypothermia, elicits substance P release from rat C-fiber neurons in primary culture. *J Clin Invest* 1995; 95:2359–66.
- 17 Anderson SD, Brannan J, Spring J, et al. A new method for bronchial provocation testing in asthmatic subjects using a dry powder of mannitol. *Am J Respir Crit Care Med* 1997; 156:758–65.
- 18 Brannan JD, Koskela H, Anderson SD, Chew N. Responsiveness to mannitol in asthmatic subjects with exercise- and hyperventilation-induced asthma. *Am J Respir Crit Care Med* 1998; 158:1120–6.
- 19 Naclerio RM, Meier HL, Kagey-Sobotka A, et al. Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1983; 128:597–602.
- 20 Hunter JA, Finkbeiner WE, Nadel JA, Goetzl EJ, Holzman MJ. Predominant generation of 15-lipoxygenase metabolites of arachidonic acid by epithelial cells from human trachea. *Proc Natl Acad Sci USA* 1985; 82:4633–7.
- 21 Henke D, Danilowicz RM, Curtis JF, Boucher RC, Eling TE. Metabolism of arachidonic acid by human nasal and bronchial epithelial cells. *Arch Biochem Biophys* 1988; 267: 426–36.
- 22 Kumlin M, Ohlson E, Björck T, et al. 15 (S)-hydroxyeicosatetraenoic acid (15-HETE) is the major arachidonic acid metabolite in human bronchi. *Adv Prost Thromb Leuk Res* 1990; 21:441–4.
- 23 Schwartz LB, Lewis RA, Austen KF. Tryptase from human pulmonary mast cells. *J Biol Chem* 1981; 256:11939–43.
- 24 Baraniuk JN. Neuropeptides. *Am J Rhinol* 1998; 12:9–16.
- 25 Svensson C, Andersson M, Greiff L, Alkner U, Persson CG.

- Exudative hyperresponsiveness of the airway microcirculation in seasonal allergic rhinitis. *Clin Exp Allergy* 1995; 25:942–50.
- 26 Holtzman MJ, Pentland A, Baenziger NL, Hansbrough JR. Heterogeneity of cellular expression of arachidonate 15-lipoxygenase: implications for biological activity. *Biochim Biophys Acta* 1989; 1003:204–8.
- 27 Souques F, Crampette L, Mondain M, et al. Stimulation of dispersed nasal polyp cells by hyperosmolar solutions. *J Allergy Clin Immunol* 1995; 96:980–5.
- 28 Willumsen NJ, Davis CW, Boucher RC. Selective response of human airway epithelia to luminal but not serosal solution hypertonicity. Possible role for proximal airway epithelia as an osmolality transducer. *J Clin Invest* 1994; 94:779–87.
- 29 Makker HK, Walls AF, Goulding D, et al. Airway effects of local challenge with hypertonic saline in exercise-induced asthma. *Am J Respir Crit Care Med* 1994; 149:1012–9.
- 30 Makker HK, Springall DR, Redington AE, et al. Airway endothelin levels in asthma: influence of endobronchial hypertonic saline challenge. *Clin Exp Allergy* 1999; 29:241–7.
- 31 Togias AG, Lai G, Pholip G. Hyperosmolar nasal challenge stimulates reflex nasal secretion (Abstract). *J Allergy Clin Immunol* 1994; 93:A217.
- 32 Lai G, Philip G, Togias A. The nasal response to hyperosmolar saline (HS) is inhibited by capsaicin treatment (Abstract). *J Allergy Clin Immunol* 1996; 97:A431.
- 33 Rinder J, Stjärne P, Lundberg JM. Capsaicin de-sensitization of the human nasal mucosa reduces pain and vascular effects of the lactic acid and hypertonic saline. *Rhinology* 1994; 32:173–8.
- 34 Greiff L, Wollmer P, Svensson C, Andersson M, Alkner U, Persson CGA. Effects of hypertonic saline on mucosal exudation of plasma in human airways (Abstract). *Allergy Clin Immunol News* 1994; 76 (Suppl. 2):76.
- 35 Greiff L, Wollmer P, Erjefalt I, Andersson M, Pipkorn U, Persson CG. Effects of nicotine on the human nasal mucosa. *Thorax* 1993; 48:651–5.
- 36 Halldorsdottir H, Greiff L, Wollmer P, et al. Effects of inhaled histamine, methacholine and capsaicin on sputum levels of alpha 2-macroglobulin. *Thorax* 1997; 52:964–8.
- 37 Eggleston PA, Hendley JO, Gwaltney JM Jr, Eggleston AW, Leavell BS Jr. Histamine in nasal secretions. *Int Arch Allergy Appl Immunol* 1978; 57:193–200.
- 38 Schultz K-D, Furkert J, O'Connor A, et al. Determination of substance P in human nasal lavage fluid. *Neuropeptides* 1996; 30:117–24.
- 39 Greiff L, Pipkorn U, Alkner U, Persson CGA. The 'nasal pool' device applies controlled concentrations of solutions on human nasal airway mucosa and samples its surface exudations/secretions. *Clin Exp Allergy* 1990; 20:253–9.