Hypertonic saline increases secretory and exudative responsiveness of human nasal airway *in vivo*

L. Greiff*, M. Andersson*, P. Wollmer[#], C.G.A. Persson[†]

Hypertonic saline increases secretory and exudative responsiveness of human nasal airway in vivo. L. Greiff, M. Andersson, P. Wollmer, C.G.A. Persson. ©ERS Journals Ltd 2003.

ABSTRACT: Hypertonic saline (HS) is used in sputum induction studies. However, little is known about the physiological effects of HS on human airways *in vivo*. The present study takes advantage of the fact that the airway effects of topical challenges may be accurately examined in the readily accessible nasal airway. The present study specifically examines whether exposure to HS affects histamine challenge-induced exudation of plasma (α_2 -macroglobulin) and methacholine-induced secretion of mucin (fucose).

Isotonic saline and HS (27 and 45 g·L⁻¹), with and without concomitant histamine challenges, and with and without preceding methacholine challenges, were administered onto the nasal mucosa in 16 healthy subjects. Lavage fluid levels of α_2 -macroglobulin and fucose were analysed.

Histamine produced a significant mucosal output of plasma (α_2 -macroglobulin). HS itself did not evoke exudation of α_2 -macroglobulin, but it significantly increased the plasma exudation effect of histamine. Methacholine produced a significant nasal mucosal output of fucose. HS also increased the mucin secretion (fucose), and it enhanced the secretory effect of methacholine.

The authors concluded that hypertonic saline alone evokes mucinous secretion in human nasal airways *in vivo* and that it also enhances the exudative and secretory effects of histamine and methacholine, respectively. Through different mechanisms the HS exposure may also improve the recovery of soluble indices in human nasal airways. Whether or not the present findings are translatable to human bronchial airways remains to be examined.

Eur Respir J 2003; 21: 308–312.

Depts of *Otorhinolaryngology, Head & Neck Surgery and [†]Clinical Pharmacology, University Hospital, Lund, and [#]Dept of Clinical Physiology, University Hospital, Malmö, Sweden.

Correspondence: L. Greiff, Dept of Otorhinolaryngology, Head & Neck Surgery, University Hospital, S-221 85 Lund, Sweden.

Fax: 46 462110968

E-mail: lennart.greiff@skane.se

Keywords: Airway exudation inflammation methacholine rhinitis secretion

Received: November 16 2001

Accepted after revision: August 6 2002

The study was supported by the Swedish Medical Research Council and the Medical Faculty of Lund University.

Inhalation of hypertonic saline (HS) is commonly used to induce sputum for diagnostic and research purposes. In patients with asthma, inhalation of this agent may produce minor bronchoconstriction [1], but sputum induction is considered a safe and exceedingly useful technique. However, little is actually known about bronchial end-organ effects of HS. For example, possibly reflecting the difficulties involved in obtaining true baseline conditions, effects of HS on the secretory and exudative capacity of the mucosa have not been well examined in the human bronchi. By contrast, in the accessible human nasal airway baseline conditions can readily be created from which changes induced by provocations and disease processes may accurately be determined [2]. Furthermore, information obtained in the nasal airway may be relevant to the bronchial airways [2].

Extravasation and airway luminal entry of plasma is a feature of both rhinitis and asthma, but may also be produced acutely in the healthy airway in response to inflammatory mediators and insults [3]. Secretion is an acknowledged mucosal defence mechanism in health and it is commonly exaggerated in airway disease [4]. In a recent study of nasal airway responses

[5], HS challenge (100 μ L⁻¹ of a 27–216 g·L⁻¹ solution) was reported to produce nasal symptoms as well as mucosal output of secretory products (lactoferrin and mucoglycoprotein markers: 7F10-mucin and Alcian blue stain). Nasal lavage fluid levels of immunoglobulin (Ig) G, possibly reflecting plasma exudation, were unaffected [5]. These results indicate that HS itself may induce secretion. It would be of interest to examine if HS could affect the secretory or exudative responsiveness of the airway mucosa.

In the present study, involving healthy subjects, the nose has been used to examine challenge-induced responses of the human airway mucosa *in vivo*. Therefore determining whether nasal lavages with HS, compared to isotonic saline, alters the effects of exudative histamine challenges and secretory methacholine challenges. Fucose and α_2 -macroglobulin were used as indices of mucinous secretion and plasma exudation, respectively.

Material and methods

A total of 16 healthy subjects (mean age 25, range 21–29) were examined. The subjects had no history

of allergic airway disease, chronic airway disease, or infectious nasal disease in the preceding two months of the study. The absence of an allergic airway disease was verified by a negative skin-prick test. The subjects had no ongoing drug treatment. The study was approved by the ethics committee, and informed consent was obtained. The study was conducted according to the Declaration of Helsinki.

Each of the study subjects were examined on six different study days (fig. 1). The study days were separated by at least 72 h. At each occasion, the visit started with a 10 min lavage that was carried out using the nasal pool-technique [6]. At each visit a set amount of saline was used, visits 1 and 4 used $9 \text{ g}\cdot\text{L}^{-1}$, visits 2 and 5 used $27 \text{ g}\cdot\text{L}^{-1}$ and visits 3 and 6 used $45 \text{ g}\cdot\text{L}^{-1}$. At visits 1–3, a second 10 min lavage, which also contained histamine ($400 \mu\text{g}\cdot\text{mL}^{-1}$), was carried out 20 min after the first lavage had been completed. At visits 4–6 a nasal challenge with methacholine ($25 \text{ mg}\cdot 100 \mu\text{L}^{-1}$) was carried out. A nasal spray device was used 10 min after the first lavage to administer the methacholine. A further 10 min after this was performed another lavage was carried out using the nasal pool technique. Any "secretion" produced during the 10 min period between the methacholine challenge and the following lavage was collected, using a funnel attached to a test tube, and mixed with the subsequently obtained lavage fluid.

The α_2 -macroglobulin was measured using a radio-immunoassay sensitive to $7.8 \text{ ng}\cdot\text{mL}^{-1}$ [7]. The intra- and inter-assay coefficients of variation are 3.8–6.0% and 3.1–7.2%, respectively. Fucose was measured using parallel ligand-exchange chromatography and fluorescence detection sensitive to $5.0 \mu\text{M}$ [8]. The intra- and inter-assay coefficients of the variation were 15–25% and 20–35%, respectively.

Differences in nasal lavage fluid levels of α_2 -macroglobulin and fucose were examined using the Friedman-test and the Wilcoxon signed rank test.

When $p \leq 0.05$ the result were considered significant. Data are presented as mean $\pm$ SEM.

Results

The HS produced minor rhinitis symptoms as well as a minor smart/pain response. The cumulative symptom scores calculated from recordings of sneezes, secretion, and blockage (range 0–9) were 0.5 ± 0.1 , 0.6 ± 0.2 ($p=0.4$), and 0.6 ± 0.1 ($p=0.8$) for isotonic saline and for HS $27 \text{ g}\cdot\text{L}^{-1}$ and $45 \text{ g}\cdot\text{L}^{-1}$, respectively. The corresponding pain scores were 0 ± 0 , 0.3 ± 0.1 ($p<0.05$) and 0.6 ± 0.1 ($p<0.01$).

By adding prechallenge lavage levels at visits one and four (*i.e.* isotonic saline), at visits two and five (*i.e.* HS $27 \text{ g}\cdot\text{L}^{-1}$), and at three and six (*i.e.* HS $45 \text{ g}\cdot\text{L}^{-1}$) the effects of HS could be analysed. The results revealed that HS produced some degree of mucin secretion (fig. 2a) but no plasma exudation (fig. 2b). Histamine produced significant mucosal output of α_2 -macroglobulin ($p<0.001$, compared with prechallenge levels) at observation with isotonic saline as well as at both concentrations of HS (fig. 3). HS $45 \text{ g}\cdot\text{L}^{-1}$ significantly increased the plasma-exudation-producing effect of histamine ($p<0.05$) (fig. 3). Methacholine produced significant mucosal output of fucose ($p<0.01$ – 0.05 , compared with prechallenge levels) as determined after either concentration of HS, but not at the observation with isotonic saline ($p>0.05$) (fig. 4). HS $45 \text{ g}\cdot\text{L}^{-1}$ significantly increased the fucose-secreting effect of methacholine ($p<0.001$) (fig. 4).

Discussion

In the present study, nasal challenge with HS alone evoked global mucinous secretion, as reflected by fucose, without inducing any inflammatory plasma exudation response, as reflected by α_2 -macroglobulin.

Fig. 1. – Nasal challenge and lavage schemes with isotonic ($\square$) and hypertonic saline ($\boxtimes$: $27 \text{ g}\cdot\text{L}^{-1}$; $\blacksquare$: $45 \text{ g}\cdot\text{L}^{-1}$). At visits 1, 2 and 3, (a–c) the second of each of the paired lavages contained histamine ($400 \mu\text{g}\cdot\text{L}^{-1}$). At visits 4, 5, 6 (d–f) the second of each of the paired lavages were preceded by a nasal spray challenge with methacholine (25 mg ; arrows).

Fig. 2.—Effects of hypertonic saline (HS) on the nasal mucosal output of a) α_2 -macroglobulin b) fucose. The figure comprises observations from the first lavage obtained at each of the six visits i.e. the "baseline recordings". HS produced a marginal but statistically significant increase in mucinous secretion. **: $p < 0.01$.

Fig. 3.—The effects of hypertonic saline (HS) and histamine on the levels of α_2 -macroglobulin in the nasal lavage fluid. $\square$: without histamine; $\square$: with histamine. Histamine produced plasma exudation. HS augmented the exudative effect of histamine. *: $p < 0.05$.

Furthermore, it was demonstrated that nasal histamine-/methacholine-induced plasma exudation and mucinous secretion, respectively, is augmented by interaction

Fig. 4.—The effects of hypertonic saline (HS) and methacholine on the levels of fucose in nasal lavage fluids. $\square$: without methacholine; $\square$: with methacholine. Methacholine produced mucinous secretion. HS augmented the secretory effect of methacholine. ***: $p < 0.001$.

with HS on the airway mucosa. The present results provide basic information on the effects of HS on the nasal mucosa which could potentially be relevant to the bronchial airways and to the sputum induction techniques employing HS. The authors further deduce from the present findings that exposure of the airway mucosa to HS may be employed to improve the recovery of soluble airway indices.

Fucose, a carbohydrate present in glycoproteins of mucins, is present in airway goblet cells as well as in mucosal and submucosal airway glands [9, 10]. The present authors have previously demonstrated that fucose is secreted by the human nasal mucosa in response to a range of nasal challenges including histamine, methacholine, and sensory nerve irritants (capsaicin, nicotine, and benzalkonium chloride) [11, 12] (unpublished data.). Increased levels of fucose have also been demonstrated in chronic bronchitis, "immotile cilia syndrome" and "panbronchiolitis" [13, 14]. In the present study, fucose was used as a global mucin secretion marker. In this respect, it is specific since it is locally produced and the contribution of other sources such as circulating plasma and inflammatory cells are likely to be negligible [15, 16]. Determination of fucose is of interest since mucins contribute significantly to the properties of airway mucosal surface liquids in health and disease [4].

In agreement with the observations by BARANIUK *et al.* [5] HS produced some degree of nasal symptoms in the present study. In further agreement with BARANIUK *et al.* [5], who employed 7F10-mucin and Alcian blue-stain as mucin secretion markers, HS in this study increased the nasal mucosal output of fucose. In addition, it was demonstrated that addition of hypertonic nasal lavage fluids markedly augments the mucinous secretion that is evoked by methacholine challenges. BARANIUK *et al.* [5] suggest that HS-induced mucin secretion reflects sensory

nerve irritation resulting in local and spinal reflexes with a local release of substance P (and likely acetylcholine). The authors agree that a similar mechanism may be involved in the present secretory responses to HS, and possibly contributing also to the HS-induced responsiveness to methacholine challenges. Indeed, sensory nerve irritation of the human nasal mucosa by either nicotine or capsaicin evokes significant secretion of fucose [11] (unpublished data). However, it cannot be excluded that mucosal exposure to HS may induce a detethering effect on mucinous secretions, making them more available to lavage fluids than at isotonic conditions.

One of the largest proteins of circulating plasma is α_2 -macroglobulin and under normal circumstances it is present in airway mucosal surface liquids in minute amounts. Since inflammatory exudation of plasma, also across an intact airway epithelial lining, is characterised by a nonsieved, bulk flux of proteins into the airway lumen, mucosal surface liquid levels of α_2 -macroglobulin would well reflect this exudation response [3, 17]. Frequently, a large plasma protein (α_2 -macroglobulin) is also a superior index to smaller proteins such as albumin and IgG that may enter the airway lumen nonspecifically [3]. In the present study, HS failed to produce plasma exudation, but exudation produced by histamine challenge was significantly increased. Since sensory nerve stimulation of the human nasal, as well as bronchial mucosa, is without plasma exudation producing effects [7, 18], it seems unlikely that sensory nerve mechanisms are involved in the increased exudative responsiveness that was induced by HS in this study. The possibility that HS evokes osmotically driven movements of solutes from the lamina propria to the airway lumen may have contributed to the present finding. However, further studies are warranted to elucidate the mechanisms involved in the present HS-induced enhanced exudative responsiveness of the airway mucosa.

The present observation that addition of HS to the nasal lavage fluid augments secretory responses to methacholine and exudative responses to histamine may be relevant to sputum techniques employing HS. This may be particularly true for plasma exudation events; thus, the current authors previously demonstrated that a variety of inflammatory mediators as well as disease factors (*e.g.* allergen) produce similar exudation responses in nasal and bronchial airways [2, 3]. Ongoing exudative responses, *e.g.* in asthma and chronic obstructive pulmonary disease, may speculatively be enhanced by the inhalation of HS. If so, this interaction may be viewed as a drawback, but it may also be an experimental advantage increasing the yield in airway luminal samples of plasma proteins and cellular products that move along with the bulk plasma [3].

The authors conclude that the topical administration of hypertonic saline to human nasal mucosa *in vivo* evokes mucinous secretion and, more importantly, that it increases secretory and exudative responses to topical challenge with methacholine and histamine, respectively.

References

1. 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: 1285–1287.
2. Persson CGA, Svensson C, Greiff L, *et al.* The use of the nose to study the inflammatory response in the respiratory tract. *Thorax* 1992; 47: 993–1000.
3. Persson CGA, Erjefält JS, Greiff L, *et al.* Plasma-derived proteins in airway defence, disease and repair of epithelial injury. *Eur Respir J* 1998; 11: 958–970.
4. Rogers DF. Airway goblet cells: responsive and adaptable frontline-defenders. *Eur Respir J* 1994; 7: 1690–1706.
5. Baraniuk JN, Ali M, Yuta A, Fang SY, Naranch K. Hypertonic saline nasal provocation stimulates nociceptive nerves, substance P release, and glandular mucous exocytosis in normal humans. *Am J Respir Crit Care Med* 1999; 160: 655–662.
6. Greiff L, Andersson M, Persson CGA. Nasal secretions/exudations: Collection and approaches to analysis. In: Rogers D, Donnelly L, eds. *Methods in molecular medicine* (Vol 56): Human airway inflammation. Totowa, Humana Press, 2001; pp. 61–73.
7. Haldorsdottir H, Greiff L, Wollmer P, Andersson M, Svensson C, Persson CGA. Effect of inhaled histamine, methacholine, and capsaicin on sputum levels of α_2 -macroglobulin. *Thorax* 1997; 52: 964–968.
8. Freney M, Irth H, Lindberg H, Alkner U, *et al.* Fast screening of fucose in airway secretions by parallel ligand-exchange chromatography in combination with post-column derivatisation and fluorescence detection. *Chromatographia* 2001; 54: 439–445.
9. Mariassy AT, Plopper CG, St. George JA, Wilson DW. Tracheobronchial epithelium of the sheep: IV. Lectin histochemical characterization of secretory epithelial cells. *Anat Res* 1988; 222: 49–59.
10. Castells MT, Ballesta J, Madrid JF, Aviles M, Martinez-Menarguez JA. Characterization of glycoconjugates in developing rat respiratory system by means of conventional and lectin immunocytochemistry. *Histochemistry* 1991; 95: 419–426.
11. Greiff L, Wollmer P, Erjefält I, Andersson M, Pipkorn U, Persson CGA. Effects of nicotine on the human nasal mucosa. *Thorax* 1993; 48: 651–655.
12. Storaas T, Andersson M, Persson CGA, Steinsvåg SK, Marko-Varga G, Greiff L. Effects of benzalkonium chloride on innate immunity physiology of the human nasal mucosa *in vivo*. *Laryngoscope* 2000; 110: 1543–1547.
13. Kurashima K, Fujimura M, Hoyano Y, Takemura K, Matsuda T. Effect of thromboxane A₂ synthetase inhibitor, OXY-046, on sputum in chronic bronchitis and diffuse panbronchiolitis. *Eur Respir J* 1995; 8: 1705–1711.
14. Nakamura S, Kawakami M, Mikami M, Yamanaka E. Physicochemical properties of sputum from patients with immotile cilia syndrome. *Nippon Kyobu Shikkan Gakkai Zasshi* 1993; 31: 1553–1559.
15. Lopez-Vidriero M. Individual and group correlations of sputum viscosity and airways obstruction. *Bull Physiopathol Respir (Nancy)* 1973; 9: 339–347.
16. Keal EE, Reid L. Pathologic alterations in mucus in asthma within and without the cell. In:

- Stein M, ed. New directions in asthma. Park Ridge, American College of Chest Physicians, 1975; pp. 223–239.
17. Greiff L, Andersson M, Erjefält JS, Svensson C, Persson CG. Loss of size-selectivity at histamine-induced exudation of plasma proteins in atopic nasal airways. *Clin Physiol Func Imag* 2002; 22: 28–31.
 18. Greiff L, Svensson C, Andersson M, Persson CGA. Effects of topical capsaicin in seasonal allergic rhinitis. *Thorax* 1995; 50: 225–22.