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Effects of experimental changes in nasal airway pressure on mucosal output of plasma

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Summary

Microvascular extravasation, lamina propria flooding and luminal entry of plasma are key features of airway inflammation. We have suggested that the extravasated plasma moves across the epithelial lining along hydrostatic pressure gradients. The present study, involving healthy subjects, tests this hypothesis by examining effects of experimentally applied negative and positive luminal pressures on nasal output of plasma at baseline and at histamine-induced plasma exudation. The negative (-10 cmH_2O) and positive (10 cmH_2O) pressures were applied for 10 min after nasal spray administrations of diluent (saline) and histamine (0·5 mg). The mucosa was then lavaged and the lavage fluid levels of α_2-macroglobulin were measured as index of plasma exudation. Nasal administrations of diluent and histamine (0·5 mg) were also carried out without any pressure applications. Histamine produced significant mucosal exudation of plasma. The negative luminal pressure augmented this response significantly as well as the baseline appearance of α_2-macroglobulin in mucosal surface liquids. We conclude that extravasated plasma may be moved across the epithelium by a hydrostatic pressure-operated epithelial mechanism.

Introduction

Microvascular extravasation, lamina propria flooding and luminal entry of bulk plasma are key features of airway inflammation (Persson *et al.*, 1998). Accordingly, topical airway applications of various inflammatory mediators, including histamine, produce increased levels of different-sized plasma proteins in airway mucosal surface liquids (Greiff *et al.*, 2002). Also, this response has been well documented in airways disease characterized by inflammation, such as allergic rhinitis (e.g. Naclerio *et al.*, 1983) and asthma (e.g. van der Graaf *et al.*, 1991). The most important implication may be that adhesive, leucocyte-activating, growth-factor active, complement-active, and otherwise biologically active plasma proteins will promptly operate in and on an insulted but still intact airway mucosa (Persson *et al.*, 1998).

Plasma extravasation is induced by actions of inflammatory mediators on endothelial cells of subepithelial, postcapillary venules (Majno & Palade, 1961; Majno *et al.*, 1961): These cells are separated and plasma is extravasated into the lamina propria. We have demonstrated that the plasma exudate rapidly enters the airway lumen, and we have suggested that plasma also moves across the epithelial lining aided by a pressure-operated mechanism, allowing epithelial cells to separate transiently and plasma to move into the airway lumen via a paracellular routes (Erjefält & Persson, 1989; Persson *et al.*, 1998). This hypothesis is supported by observations *in vitro* demonstrating that application of a slight hydrostatic pressure (5 cmH_2O) on the serosal aspect of guinea-pig tracheal tube preparations readily produces luminal entry of macromolecules (Persson *et al.*, 1990; Gustafsson & Persson, 1991).

In the present study, involving healthy subjects, we have tested the above hypothesis by examining whether or not experimentally applied negative and positive nasal airway luminal pressures affect the nasal mucosal output of plasma at baseline and at histamine-induced plasma exudation. We have thus employed a lavage technique and we have monitored the lavage fluid levels of the plasma protein α_2-macroglobulin (mol. wt 720 kDa) as index of luminal entry of bulk plasma.

Material and methods

Subjects

Fifteen healthy subjects (aged 22–34 years, mean age 26 years) participated in the study. The subjects had no history of chronic or recent nasal disease and no history of ongoing or recent drug treatment. The study was approved by the local research ethics committee and informed consent was obtained.

Study protocol

The subjects were examined on four occasions:

(i) Diluent challenge (saline) without subsequent negative nasal pressure challenge.
(ii) Histamine (0·5 mg) challenge without subsequent negative nasal pressure challenge.
(iii) Diluent challenge with subsequent negative nasal pressure challenge (−10 cmH_2O).
(iv) Histamine (0·5 mg) challenge with subsequent negative nasal pressure challenge (−10 cmH_2O).

Ten of these subjects were also examined on two additional occasions:

(i) Diluent challenge with subsequent positive nasal pressure challenge (10 cmH_2O).
(ii) Histamine (0·5 mg) challenge with subsequent positive nasal pressure challenge (10 cmH_2O).

Nasal challenges with either isotonic saline or histamine (5·0 mg × ml^{-1}) in isotonic saline were carried out using a nasal spray-device. The spray-device delivered 50 μl per actuation and two actuations were given to the right nasal cavity at each occasion. The delivered dose of histamine was thus 0·5 mg.

Negative nasal airway pressure was accomplished by connecting the subject's nose via an adapter to a flask with controlled pressure. The pressure in the flask was generated by applying suction to the flask and controlled by means of leakage through a water lock. The negative pressure applied was −10 cmH_2O. The nasal adapter was inserted into one nostril by the subject who was instructed to manually close the other nostril and to voluntarily close the soft palate. The negative nasal pressure was applied approximately 1 min after the saline and histamine challenges and maintained for 10 min. The subject was instructed to breathe through the mouth during this procedure.

A positive nasal pressure was applied using a continuous positive airway pressure (CPAP) device fitted with a nasal mask and set to exert a pressure of 10 cmH_2O. The mask was applied approximately 1 min after the saline and histamine challenges and maintained for 10 min. The subject was instructed to breath through the nose with the mouth closed.

A nasal pool-device (Greiff *et al.*, 1990), a compressible plastic container equipped with a nasal adapter, was used for lavages of the nasal mucosa. The adapter was inserted into the right nostril and the container is compressed by the sitting subject leaning forward in a 60° flexed neck position. The nasal pool-fluid was thus instilled in the nasal cavity and maintained in contact with the mucosal surface for 5 min. When the pressure on the device was released the fluid returned into the container. In the present study, the nasal pool-device contained 15 ml isotonic saline.

Analysis of α_2-macroglobulin

The lavage fluid levels of α_2-macroglobulin were measured using a radioimmunoassay sensitive to 10 ng × ml^{-1}. Rabbit antihuman α_2-macroglobulin (Dakopatts, Copenhagen, Denmark) was used as antiserum and human serum (Behringwerke, Marburg, Germany) as standard. Human α_2-macroglobulin (Cappel-Organon, Turnhout, Belgium) was iodinated using the lactoperoxidase method. Tracer and standard (or sample) were mixed with antiserum before adding goat antirabbit antiserum (AstraZeneca, Lund, Sweden). The bound fraction was measured using a gamma counter (Pharmacia, Uppsala, Sweden). The intra- and inter-assay coefficients of variation are between 3·8–6·0 and 3·1–7·2%, respectively.

Statistics

Wilcoxon signed rank test was used to examine differences in lavage fluid concentrations of α_2-macroglobulin. A P-value less than 0·05 was considered significant. Data are presented as mean ± SEM.

Results

The baseline appearance of α_2-macroglobulin was low and consistent with previously reported baseline levels (Persson *et al.*, 1998; Greiff *et al.*, 2002). Furthermore, histamine produced an expected, marked mucosal output of α_2-macroglobulin ($P<0·001$).

The baseline appearance of α_2-macroglobulin ($P<0·01$) as well as the histamine-induced mucosal output of α_2-macroglobulin ($P<0·05$) was increased by the application of a negative luminal pressure (−10 cmH_2O) (Fig. 1).

The application of a positive nasal pressure (10 cmH_2O) reduced the baseline appearance of α_2-macroglobulin as well as the histamine-induced plasma exudation to some extent, but these changes failed to reach statistical significance (Fig. 2).

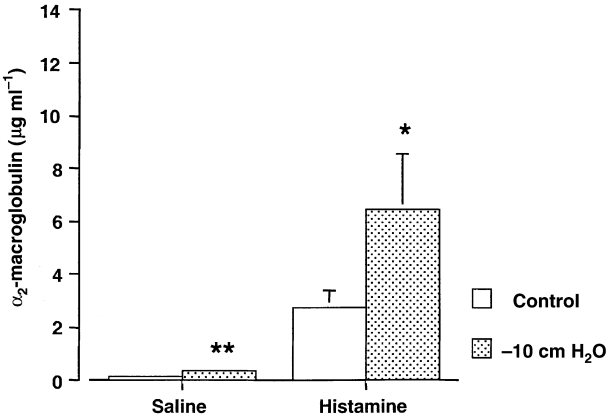

Figure 1 α_2-Macroglobulin in nasal lavage fluids obtained at baseline (saline challenge) and following challenge with histamine in the absence and presence of a negative pressure challenge. Histamine produced a marked plasma exudation response (significance levels are give elsewhere). The negative pressure increased this response as well as the baseline appearance of α_2-macroglobulin (*denote $P<0·05$ and **denote $P<0·01$).

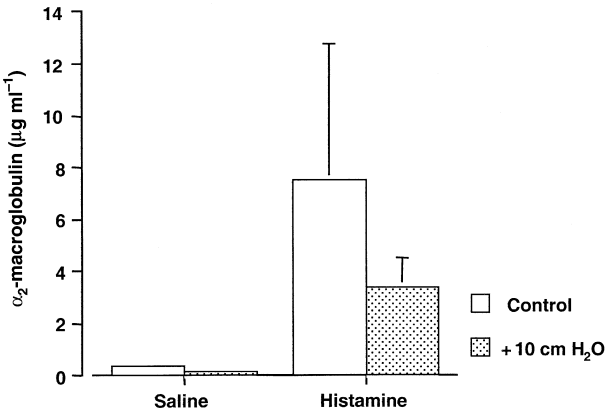

Figure 2 α_2-Macroglobulin in nasal lavage fluids obtained at baseline (saline challenge) and following challenge with histamine in the absence and presence of a positive pressure challenge. The positive pressure decreased the baseline appearance of α_2-macroglobulin and the exudative response to histamine, but these trends failed to reach statistical significance. However, in subjects that underwent negative as well as positive pressure challenges, the negative pressure challenge was associated with greater lavage fluid levels of α_2-macroglobulin at diluent challenge ($P<0{\cdot}05$) as well as at histamine challenge ($P<0{\cdot}05$) compared with the positive pressure challenge.

In the subgroup of subjects that underwent negative as well as positive pressure challenges ($n = 10$), the negative pressure challenge was associated with greater lavage fluid levels of α_2-macroglobulin after the diluent challenge ($P<0{\cdot}05$) as well as after the histamine challenge ($P<0{\cdot}05$) compared with the positive pressure challenge.

Discussion

The present study has demonstrated that nasal mucosal output of plasma, as indicated by lavage fluid levels of α_2-macroglobulin, is sensitive to experimental changes in the nasal airway (luminal) pressure. This finding is in agreement with our hypothesis that airway luminal entry of extravasated plasma involves a hydrostatic pressure-operated, valve-like epithelial mechanism.

After the extravasation of plasma from the subepithelial microcirculation, there is a phase when the surrounding lamina propria is flooded with plasma proteins. Promptly, the plasma exudate then moves up between and around the epithelial lining cells, and makes ubiquitous paracellular pathways into the airway lumen (Erjefält *et al.*, 1995). Although this process involves movement of bulk plasma, including the largest plasma proteins such as α_2-macroglobulin (this study), the luminal entry may not damage the epithelial lining (Erjefält *et al.*, 1995), nor does it increase mucosal absorption, i.e. the perviousness of the epithelium to luminal solutes (Greiff *et al.*, 1991a,b; Persson *et al.*, 1998). We have previously suggested that this unidirectional increase in the outward permeability of the epithelial lining reflects a hydrostatic pressure-operated valve-like mechanism whereby a slight increase in the subepithelial hydrostatic pressure, created by the plasma exudate itself and its attracted fluids, allows epithelial cells to transiently separate. In the present study, the observation that luminal entry of plasma proteins, at baseline and at exudative conditions, is affected by hydrostatic pressure changes supports our hypothesis that luminal entry of plasma proteins occurs along hydrostatic pressure gradients.

α_2-Macroglobulin represents a specific binding capacity of plasma proteins (Peterson & Venge, 1987). The present data on luminal entry of bulk plasma (α_2-macroglobulin) is in agreement with the possibility that extravasated plasma may bind various pro-inflammatory factors, e.g. eosinophil cationic protein (ECP), occurring in the tissue, and move them to the airway surface for clearance through mucociliary transport and other mechanisms. Rinsing mucosal interstices, including the para-epithelial spaces, may be a component of the innate immunity role of plasma exudation. However, the most important implication may be that adhesive, leucocyte-activating, growth-factor active, complement active, or otherwise biologically active plasma proteins will promptly operate not only in the mucosal tissue but also on the surface of an insulted but still intact airway mucosa.

We conclude that experimental application of a negative luminal airway pressure augments baseline appearance of plasma proteins in nasal mucosal surface liquids as well as histamine-induced mucosal exudation of bulk plasma. This finding supports the view that extravasated plasma may move across the epithelium along hydrostatic pressure gradients.

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