Inhalation of hypertonic saline aerosol has been shown to increase mucociliary clearance (MCC) in patients with bronchitis (1) and cystic fibrosis (2) who have impaired baseline mucociliary clearance. Although, these studies suggest that hypertonic saline stimulates the mucociliary system, there are only a few studies that have reported the effect of hypertonic saline on MCC in healthy subjects with normal baseline mucociliary clearance.

Nasal nitric oxide is present in high concentration in the upper respiratory tract. The main source of this gaseous molecule is the paranasal sinus epithelium. The physiological role of this mediator is to contribute to local host defence, modulate ciliary motility and serve as an aerocrine mediator in helping to maintain adequate ventilation-perfusion matching in the lung. Abnormal values of nasal NO (nNO) have been reported in different physiological and pathological conditions. Reduced nNO values have been recorded in subjects with acute and chronic sinusitis, cystic fibrosis and nasal polyps. Particularly low concentrations have been described in children with primary ciliary dyskinesia (3). Nowadays, there are no reported studies about the effect of hypertonic saline inhalation on nNO levels in healthy subjects.

The aim of the presented study was to investigate the effect of a hyperosmolar stimulus on MCC and nNO in healthy subjects.

Informed written consent was obtained from all subjects before they participated in the study.

Subjects

43 healthy volunteers: 18 men aged 20.6±1.2 years, average height 183.5±7.4 cm, average weight 77.8±9.6 kg and 25 women aged 21.2±1.5 years, average height 170.8±7.1 cm, average weight 57.1±6.2 kg, took part in the study. None of the subjects had a history of cigarette smoking and none had an upper or lower respiratory tract infection within 3 weeks prior to or during the study. Subjects had no history of asthma, allergic or chronic rhinitis, atopic eczema or other respiratory diseases.

Measurement of nasal nitric oxide

Nasal NO was measured by a chemiluminescence analyser NIOX® Nitric Oxide Monitoring System, Aerocrine, Sweden at a flow rate of 5 ml/s, using the method of direct sampling from the nose during breath holding which is the preferential technique, as breath holding keeps the soft palate closed, hence preventing mixing of nasal NO with lower airway NO. Nasal nitric oxide was measured with a probe inserted into one of the nostrils, whilst the subject breath holds, i.e. with no active exhalation (4). The breath was held for a minimum of 20
seconds. Subjects were relaxed during the breath holding manoeuvre and maintained a full inspiratory position, with the mouth closed without straining. Closure of the soft palate will allow analysis of the local NO concentration, with free flow of ambient air subsequently directed into the analyser. The value of the last plateau part of the trace of nasal NO was recorded (4). The subjects were asked to perform three measurements, the mean of these measurements being taken as the level of NO in the nose. The analyser was calibrated weekly using certified NO gas.

**Measurement of mucociliary clearance**

We used the saccharin test for evaluating mucociliary clearance (MCC). A particle of sodium saccharine was placed on the surface of the inferior nasal concha, 1 cm behind its head to avoid the area of squamous epithelium. The participants remained seated with their head tipped slightly forward while breathing normally (not forced), without sneezing or blowing their nose, and without taking any substances that might interfere with the test. They were told to indicate when they noted any particular taste. The actual taste they were to expect was not specified in order to avoid false positives. The saccharine particle was carried by means of ciliary transport along the entire nostril until it reached the oropharynx, whereupon a characteristic sweet taste could be perceived. The time was recorded in seconds using a stopwatch. The nostril with lower resistance to physiological airflow confirmed by rhinomanometry was chosen for measurement of MCC.

**Study design**

Nasal nitric oxide was measured in the right and left nostrils. Then the rhinomanometry was performed and mucociliary clearance was measured in the nostril with normal nasal airflow and pressure. Then subjects sprayed one puff of hypertonic saline Sinomarin® (2.3% solution of NaCl) into each nostril. After 30 minutes, measurement of nasal nitric oxide in both nostrils and duration of mucociliary transport in the same nostril as the first time was repeated.

**Statistical analysis**

Data of nasal nitric oxide are expressed as a median and interquartile range (IQR) in parts per billion (ppb). The duration of mucociliary transport is expressed as a median and interquartile range in seconds (s). Comparison of nNO values and time of MCC was performed using the Wilcoxon test.

**RESULTS**

The median level of nNO before inhalation of hypertonic saline in the right nostril (R-nNO) was 806 ppb, IQR 337.6 and 854 ppb, IQR 295.8 in the left nostril (L-nNO). There was no significant difference between the right and left nostril (p>0.05, Wilcoxon T-test).

The median level of nNO after inhalation of hypertonic saline in the right nostril (R-nNO) was 841.8 ppb, IQR 342.3 and 897.4 ppb, IQR 304.1 in the left nostril (L-nNO). There was also no significant difference between the right and left nostril (p>0.05, Wilcoxon T-test) (Fig. 1).

We found that levels of nNO before inhalation of hypertonic saline were significantly lower than levels after inhalation of hypertonic saline in each nostril (p<0.05, Wilcoxon T-test) (Fig. 2).

The median range of the duration of mucociliary transport before inhalation of hypertonic saline was 507 s, IQR 233 and 360 s, IQR 238 after inhalation of aerosol (Fig. 3).

We found a significant decrease (p<0.0001, Wilcoxon T-test) in the time of mucociliary transport after inhalation of aerosol, thus the mucociliary clearance significantly increased. There was no correlation found between nNO and MCC.
DISCUSSION

This study demonstrates that inhalation of hypertonic saline aerosol increases MCC and levels of nNO in healthy subjects. However, the difference between baseline and post-inhalation values of nNO seems to be discrete, 806 ppb, IQR 337.6 vs. 841.8 ppb, IQR 342.3 and 854 ppb, IQR 295.8 vs. 897.4 ppb, IQR 304.1. Nevertheless, we have confirmed a statistically significant difference. Factors which specifically affect nasal NO are not well defined and are still being discussed. We have attempted to exclude the following known factors influencing nNO levels in our study, therefore we are inclined to accept, overall, our results. It is conceivable that extraneous NO may influence nasal physiology, but more importantly, reduce the

Fig. 2. Comparison of nNO levels in right nostril (R-nNO) before and after inhalation of hypertonic saline and comparison of nNO levels in left nostril (L-nNO) before and after inhalation of hypertonic saline (the boxes represent median of nNO value, bottom and top of the box is lower and upper quartile, whiskers extend out to the data's maximum and minimum value, the values represented by the circles are outliers, * p<0.05).

Fig. 3. Comparison of MCC before and after inhalation of hypertonic saline (the boxes represent median of nNO value, bottom and top of the box is lower and upper quartile, whiskers extend out to the data's maximum and minimum value, the values represented by the circles are outliers, **p<0.001).
increase in $\text{Ca}^{2+}$ stimulates the ciliary beat frequency, possibly by release from intracellular stores (26). There is evidence that an increase in nasal NO output might be volume-dependent, provided a true steady-state plateau was achieved, in one study (10), but has been reported to be volume-dependent at low transnasal flows in another (11), possibly because of changes in nasal aerodynamics (12). Medications have been shown to affect NO. Some antibiotic classes such as macrolides have been found to have a number of potential immunomodulatory abilities to suppress the production of proinflammatory cytokines, decrease mucus synthesis and promote inflammatory cell apoptosis in bronchial epithelium (13), but our subjects did not use any medications at least one month before the study. Smoking causes a small decrease in nasal NO, but our subjects were all non-smokers.

We did not find any studies which studied the relationship between nasal NO and MCC neither in animal models, nor in human beings. In a study by Daviskas et al., inhalation of hypertonic saline aerosol enhances mucociliary clearance in asthmatic and healthy subjects, which is in accordance with our results (14). According to Robinson et al., hypertonic saline significantly improves mucociliary clearance in patients with cystic fibrosis (15). Measurement of nasal nitric oxide is still a subject of research. It has recently become known that bioavailability of nitric oxide is in relationship with glutathione levels (16) and nNO concentrations are increased in asthma, allergic rhinitis and viral respiratory infections and reduced in sinusitis, cystic fibrosis, primary ciliary dyskinesia and diffuse panbronchiolitis. The most comprehensive and significant changes in nNO concentrations in relation to normal values can be documented in patients with primary ciliary dyskinesia (17). However, nasal nitric oxide levels were not studied in relationship to hypertonic saline inhalation.

The increase in MCC is most likely to be due to mediators released in response to hyperosmolarity of the airway surface liquid, because exposure of the human airways to a hyperosmolar stimulus causes release of histamine, prostaglandin E$_2$ (PGE$_2$) and leukotriene C4 from the mast cells (18), and possibly neuropeptides (e.g. substance P) from sensory nerves (19). Animal studies, in vitro and in vivo, have shown that chemical mediators and neuropeptides can stimulate ciliary activity (20, 21) by a mechanism which is not clearly understood but may involve neural stimulation of ciliary beat frequency via the cyclooxygenase pathway (21-23). Histamine has been demonstrated to increase mucociliary clearance both in asthmatic and healthy subjects (24, 25). Additionally, hyperosmolarity of the airway fluid causes an increase in $\text{Ca}^{2+}$ release from intracellular stores (26). There is evidence that an increase in $\text{Ca}^{2+}$ stimulates the ciliary beat frequency, possibly by regulating the use or availability of adenosine triphosphate (ATP) by the axoneme of the cilia (27).

There is growing evidence that NO also plays a crucial role in the upregulation of ciliary motility (28, 29), although the cellular control of ciliary function is not well understood. As the majority of respiratory epithelial cells are ciliated and contain nitric oxide synthase (NOS), it could be speculated that NO may modulate airway ciliary beating (30). Moreover, Beier et al. described increased expression of an inducible form of NO synthase (iNOS) after osmotic stimuli that lead to increased levels of nitric oxide, which could be helpful in patients with primary ciliary dyskinesia and cystic fibrosis, who have very low levels of nNO (31).

The authors conclude that nasal nitric oxide measurement is simple and non-invasive, but the techniques require a certain level of expertise and the availability of equipment used is not currently widespread. A limitation of the study is also the method of measurement of MCC by saccharin test, which is not as exact as measurement using radioaerosol.

The finding that mucociliary clearance and levels of nasal nitric oxide increases in healthy subjects after inhaling an aerosol of hypertonic saline may have practical implications, as a response to a need to clear unwanted inhaled particles. The increase in mucociliary clearance in response to inhalation of hyperosmolar saline could help to clear accumulated secretions in the airways and prevent respiratory tract infections.

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