

Hypertonic saline nasal provocation and acoustic rhinometry

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Summary

Background Hypertonic saline (HTS) acts as an airway irritant in human nasal mucosa by stimulating nociceptive nerves and glandular secretion. HTS does not change vascular permeability. In asthma, HTS causes airflow obstruction.

Objective To determine the effect of HTS on mucosal swelling using acoustic rhinometry (AcRh). Potential vasodilator effects were controlled by maximally constricting mucosal vessels with oxymetazoline (Oxy).

Method Normal subjects had AcRh before and 30 min after either 0.05% Oxy or saline (0.9% NaCl) nasal treatments. Nasal provocations followed immediately with five step-wise incremental escalating doses of HTS administered at 6-min intervals. AcRh was performed 1, 3 and 5 min after each HTS administration, and then after blowing the nose at 5 min. The minimum cross-sectional area (Amin), volume of the anterior 6 cm of nasal cavity (V6) and incremental changes from pre-drug treatment baseline levels (Δ , mean $\pm$ SEM) were calculated.

Results Oxy increased Amin by 46% ($\Delta = 0.48 \pm 0.07 \text{ cm}^2$, $P = 0.0001$) and V6 by 53% ($\Delta = 9.9 \pm 1.5 \text{ mL}$, $P < 1 \times 10^{-7}$) during the first 30 min. Saline (vehicle) treatment had no effect. The maximum HTS dose had no effect after 1 or 3 min. However, in the 4th and 5th minutes there were reductions in Amin ($\Delta = 0.07 \pm 0.03 \text{ cm}^2$, $P = 0.035$) and V6 ($\Delta = 1.57 \pm 0.42 \text{ mL}$, $P = 0.004$) with an increase in the weight of secretions ($\Delta = 700 \pm 100 \text{ mg}$, $P < 0.05$). Blowing the nose returned Amin and V6 towards baseline. Oxy had no effect on HTS-induced changes in Amin, V6, pain, rhinorrhea or weight of secretions.

Conclusion HTS induced nociceptive nerve stimulation and mucus secretion, and reduced V6 and Amin. Oxy caused vasoconstriction but did not alter HTS-induced effects. HTS may stimulate neurogenic axon response-mediated glandular secretion that contributes to perceptions of nasal obstruction in normal subjects.

Keywords glandular secretion, hypertonic saline, mucosal swelling, neurogenic inflammation, rhinitis

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Introduction

Hypertonic saline (HTS) leads to bronchial obstruction in asthmatics [1–3]. The molecular mechanism(s) are not yet fully defined, in part because of the difficulties inherent in studying human bronchial reactions *in vivo*. In contrast, the nasal mucosa is a much more accessible site for studying airway mucosal responses. HTS induces nasal discomfort, blockage, rhinorrhea and glandular secretion [4–9]. The induction of pain and the release of the neurotransmitter substance P [9] suggest that neurogenic mechanisms are activated. Neural mechanisms have been extensively studied in rodent bronchial airways where capsaicin and other nocifors lead to plasma exudation, mucosal oedema and bronchial smooth muscle contraction [10,11]. In human skin, neurogenic responses include the vasodilation and

flare of the triple response of Lewis [12]. These lines of evidence predict that HTS-induced neural responses will lead to vascular changes with airway swelling and subsequent obstruction to airflow.

This ‘neuro-vascular’ hypothesis was tested by human nasal provocation. Nasal airspace volume was measured by acoustic rhinometry (AcRh) [13–15]. Changes in this volume may reflect (i) increased mucosal thickness due to venous sinusoid engorgement, or plasma extravasation and superficial mucosal oedema, and (ii) increased mucus volume due to plasma exudation or glandular exocytosis from serous and mucous cells. The vascular and glandular sources of these mucus constituents can be determined by biochemical analysis [16]. Previous studies have demonstrated that HTS nasal provocation leads to dose-dependent increases in pain, sensations of obstruction to nasal airflow (blockage) and rhinorrhea, weight of recovered lavage fluid, and concentrations of glandular products indicating sub-mucosal gland exocytosis [4–9]. However, there were no changes in plasma protein concentrations [4–9]. The latter indicated the absence of vascular leak. As well, unilateral HTS provocations had no significant effect of ipsilateral (IL) or

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contralateral (CL) nostril AcRh measurements, suggesting the absence of vasodilatory reflexes. These unexpected findings were further examined in this manuscript.

Oxymetazoline (Oxy) was used to vasoconstrict vessels, decrease the mucosal thickness and block the capacity of vessels to dilate in response to HTS [17,18]. The effects of Oxy vs. placebo pre-treatment were compared by AcRh and weights of secretions. Bilateral HTS provocations were used to maximize the likelihood that any change in airspace volume would be detected. If HTS caused neural responses that led to vasodilation, thickening of the nasal mucosa and obstruction to airflow, then it would be possible to speculate that HTS could lead to vasodilation and obstructed airflow in bronchi. If Oxy had no effect, then we could speculate that HTS-induced neural responses did not cause significant vasodilation, and that vasodilation may be less likely to be a component of airway neural responses.

Methods

Subjects

Normal subjects gave informed consent to participate in this IRB-approved study. Exclusion criteria included active rhinitis of any type, history of allergic rhinitis or nasal polyps, upper respiratory tract infection or sinusitis in the past 6 weeks, use of antihistamines, antidepressants with anticholinergic or antihistaminic properties, oral or nasal decongestants, any formulation of glucocorticoid drug, and any chronic disease that could interfere with airway, mucosal and neural responses. Subjects were tested on two occasions at least 1 week apart and received Oxy or saline in randomized fashion.

Pre-treatment

Subjects had three baseline AcRh measurements at 5-min intervals, and then were treated with two sprays in each nostril of either Oxy 0.05% ($n = 10$) or normal saline vehicle ($n = 10$) (15-min time-point, Fig. 1). AcRh measurements were continued at 5-min intervals for 30 min. HTS provocations followed immediately (45 min).

Hypertonic saline (HTS) nasal provocation

HTS was administered bilaterally as five increasing doses given at approximately 6-min intervals (Fig. 1). The first dose was 100 μ L of normal saline ($1 \times$ NS) squirted into each nostril from a 1-mL syringe (45 min). After 5 min, subjects gently blew any secretions from their noses. Approximately 1 min later (51 min) they received the next dose of HTS: 100 μ L per nostril of $3 \times$ NS (NaCl solution with three times the tonicity of normal saline). After 5 min, subjects again blew secretions from their noses. This cycle was repeated with $6 \times$ NS, $12 \times$ NS and $24 \times$ NS.

Mock provocation

Subjects ($n = 9$) followed the timed protocol but received no pre-treatments, provocations or lavages at all. This mock provocation assessed the effects of blowing alone on nasal mucosa.

Serial saline provocation

Subjects ($n = 9$) followed the same timed protocol but received no pre-treatments. They were blinded to the nature of the provocation. Subjects received bilateral 100 μ L provocations

with $1 \times$ NS, AcRh and nasal lavage at time-points equivalent to each of the HTS provocations. This protocol assessed potential placebo or irritant effects due to repeated saline administration.

Symptoms

Five minutes after each HTS administration, subjects scored the severity of their sensations of nasal pain, blockage and drip using 10-cm linear analogue scales. The duration of the burning sensation and parasthesias were recorded.

Weights of secretions

In a separate series of provocations the weights of secretions were assessed by spraying 12 sprays of $1 \times$ NS from a 'Beconase' hand-actuated administrator at the end of 5 min, instead of performing AcRh ($n = 9$). Weights of returned lavage fluid were calculated for each 5-min period.

Acoustic rhinometry (AcRh) timing

AcRh was performed before $1 \times$ NS and each of the other HTS administrations at 45, 51, 57, 63 and 69 min. These were termed 'Pre' time-points. AcRh measurements were made 1, 3 and 5 min after each HTS administration and 30 s after blowing the nose ('Blow'; 5½ min after each administration). This measurement was followed immediately by administration of the next higher HTS dose. In this fashion, each 'Blow' measurement became the 'Pre' measurement for the next higher dose of HTS.

The Eccovision Acoustic Rhinometry System (E. Benson Hood Laboratories Inc., Pembroke, MA, USA) was used to assess the geometry of the nasal cavity. Subjects were seated in a straight-backed chair and looked forwards, keeping their head level while the AcRh tests were conducted. Subjects stopped breathing for approximately 12 s for each AcRh measurement. The acoustic wave tube with a disposable nose tip was placed over the exterior nares to form an acoustic seal. The wave tube was activated and the acoustic wave pattern recorded once it had stabilized for at least 5 s.

Wave patterns were graphically displayed as the cross-sectional area of the nasal cavity as a function of distance from the end of the wave tube. These data were analysed to show changes in: (a) the minimum cross-sectional area that corresponds to the site of maximum resistance to nasal airflow near the anterior tip of the inferior turbinate, and (b) the volume of the cavity from the site of the minimum cross-sectional area to a point 6 cm posterior. Left and right nostril results were added together to give the total minimum area (A_{min}) and volume (V_6). Changes in V_6 gave insights into changes in the degree of mucosal thickening and volume of secretions in the airway lumen. Volumes between A_{min} and points 2 cm (V_2) and 4 cm (V_4) posterior were also assessed to determine if alterations were uniform throughout the nasal cavity, or if more anterior or posterior regions had greater changes.

It was necessary to normalize the changes in V_6 that occurred following each individual HTS administration across the subject population. The net changes in V_6 between the Pre, 1, 3 and 5 min, and Blow time-points were calculated for each HTS administration. The time intervals were: '1 min-Pre'; '3 min-1 min'; '3 min-Pre'; '5 min-3 min' (to assess the 4th and 5th min); and '5 min-Pre' (to assess the overall change in V_6 after each HTS dose). Changes due to blowing were assessed by the 'Blow-5 min' calculation. For each of the time intervals,

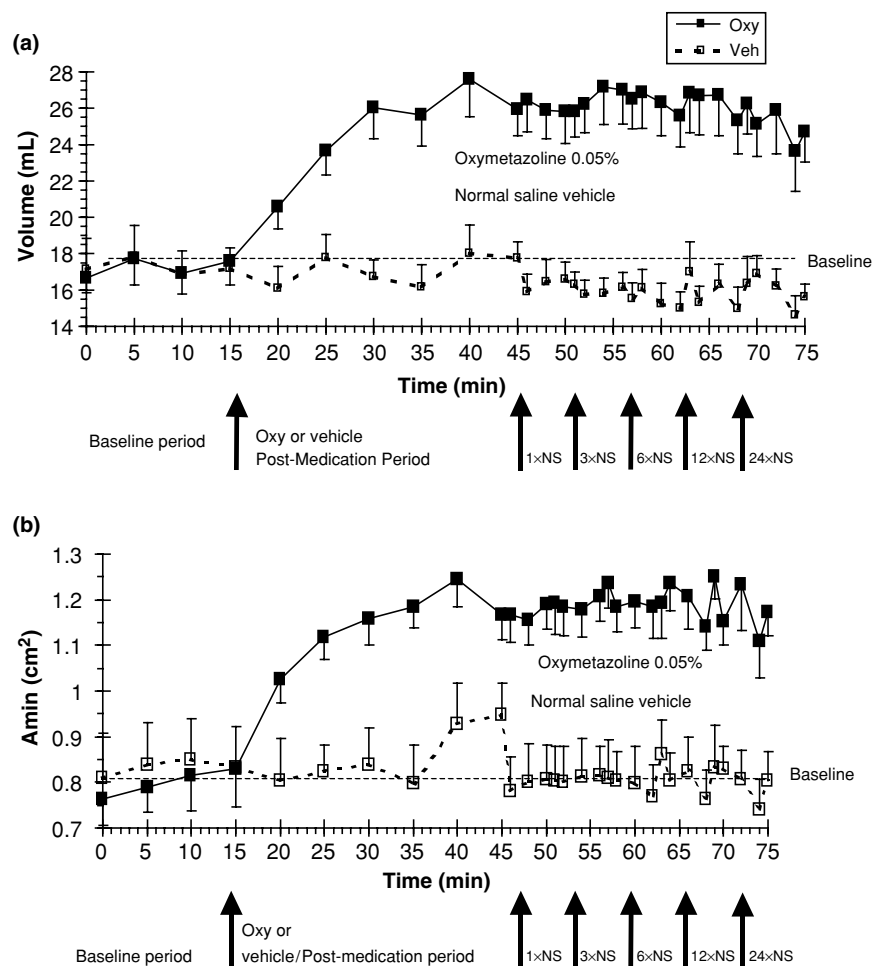


Fig. 1. Acoustic rhinometry. (a) Volume. The mean $\pm$ SEM for V6 for both nostrils are shown for oxymetazoline (filled circles) and saline (open circles) pre-treatments. The timing of measurements in the basal (0–15 min), post-medication (16–45 min) and HTS challenge periods are shown. (b) Minimum cross-sectional area. Changes in Amin are shown.

changes in V6 were plotted against HTS dose or equivalent challenge period (time-point) for the mock and serial saline provocations. In this fashion, the effects of Oxy and saline pre-treatments on the HTS-induced changes in V6 could be compared. Amin data were analysed in the same way.

Statistics

V6 and Amin measurements for baseline, 30 min (after drug/placebo administration), 'Pre', 1 min, 3 min, 5 min, 'Blow' time-points, weights and symptoms were tabulated in Excel and the mean $\pm$ standard error of the mean (SEM) determined. Data were transferred to an SPSS file for statistical analysis by ANOVA for multiple comparisons. Significant differences between 1 $\times$ NS and the various doses of HTS were compared by paired Student's *t*-tests. One-tailed tests were used for comparison of symptoms, as symptoms were near zero after 1 $\times$ NS provocations, and could only increase. Two-tailed tests were used for Amin and V6 as these could either increase or decrease after provocations. The pre-treatment effects of Oxy and saline were identified by comparing baseline and 30-min data by unpaired Student's *t*-tests. Significance was assumed for $P < 0.05$. Parametric and non-parametric tests gave equivalent significance estimates.

In order to study correlations between different derived measures (e.g. '5 min–3 min') for mock, serial saline, Oxy and vehicle

pre-treated groups, the value of 1 $\times$ NS or first provocation was subtracted from 3 $\times$, 6 $\times$, 12 $\times$ and 24 $\times$ NS data. The incremental responses (Δ) were depicted graphically and Pearson's correlations shown as r^2 .

Results

Pain

Pain was absent in the mock and serial saline nasal provocations (data not shown). Oxy and saline pre-treatments did not cause any symptoms. 1 $\times$ NS nasal provocations did not cause pain (Fig. 2a). Pain increased in dose-response fashion between 3 $\times$ NS ($P < 0.048$) and 24 $\times$ NS ($P < 0.001$) after both pre-treatments. The pain had a burning character that faded to a paresthetic sensation with time. The duration of the sensation increased in significant, dose-dependent fashion between 3 $\times$ NS ($P < 0.04$) and 24 $\times$ NS ($P < 1 \times 10^{-6}$) for both pre-treatments (Fig. 2b). However, pain duration was significantly shorter after Oxy than vehicle pre-treatment for 12 $\times$ NS ($P = 0.027$) and 24 $\times$ NS ($P = 0.015$).

Nasal blockage

The sensation of blockage was not induced by mock or serial saline nasal provocations (data not shown). Block was

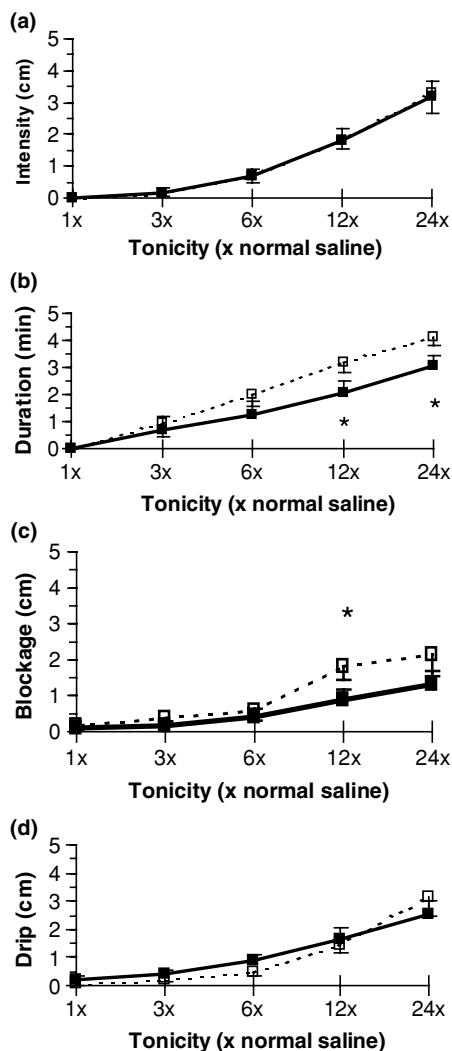


Fig. 2. Symptoms. HTS induced significant dose-dependent increases in the sensations of pain intensity (a), pain duration (b), blockage (c) and drip (d). Oxymetazoline (black squares) significantly reduced pain duration (12 and 24 × NS, $P < 0.03$) and blockage (12 × NS, $P = 0.02$) compared with saline pre-treatment (white squares). Mock provocations and serial saline provocations had no effect on symptoms (data not shown).

increased in dose-response fashion between 6 × NS ($P < 0.05$) and 24 × NS ($P < 0.01$) after both pre-treatments (Fig. 2c). Blockage was significantly lower after Oxy than saline after 12 × NS ($P = 0.02$).

Drip

Rhinorrhea began at 6 × NS ($P = 0.02$) and increased in dose-dependent fashion to 24 × NS ($P = 0.003$) (Fig. 2d). Oxy had no effect. No drip was scored during mock or serial provocations (data not shown).

Weights of recovered lavage fluid

HTS significantly increased weights after 12 × NS and 24 × NS for both Oxy and saline pre-treatments (Fig. 3). There were no differences between Oxy and saline pre-treatments at any HTS dose. This suggested that Oxy may have had no effect on the mechanisms contributing to HTS-induced secretion

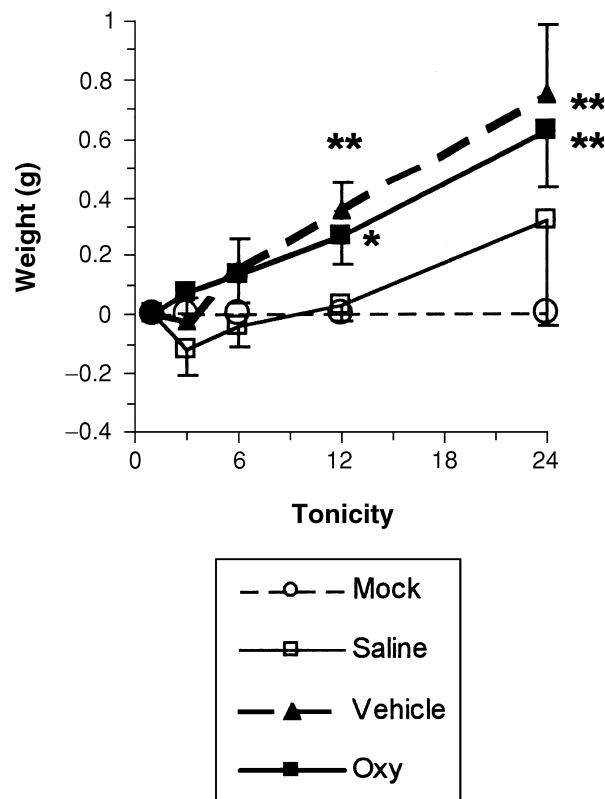


Fig. 3. Change in the weight of recovered lavage fluids from 1 × NS or first provocations. Weight (g) was plotted against HTS concentrations or the homologous serial mock or saline provocation. No secretions were generated by mock provocations (no lavage) (open circles, dashed line). Serial saline provocations with lavage also show no significant changes (open squares, solid line). HTS at 12 × NS and 24 × NS caused significant weight increases (* $P < 0.05$, ** $P < 0.01$). Weights following vehicle (solid triangles, dashed line) and Oxy (solid squares, solid line) pre-treatments were equivalent.

production. There were no changes after mock or serial saline challenges.

Effect of oxymetazoline pre-treatment on nasal airspace volume

The baseline V6 was 16.9 ± 0.1 mL (mean $\pm$ SEM of measurements between 0 and 15 min) (Fig. 1a). V6 was unchanged (16.9 ± 0.6 mL) 30 min after saline pre-treatment (45 min time-point). Oxy-pre-treated nostrils had baseline V6 of 17.2 ± 0.3 mL that rose by 53% to 26.3 ± 0.4 mL ($P < 1 \times 10^{-7}$) after 30 min. The percentage changes in V2 and V4 paralleled V6, suggesting that there was no advantage in using these anterior nasal cavity subdivision measurements (data not shown).

Effect of oxymetazoline pre-treatment on Amin

Amin was 0.80 ± 0.11 cm² before and 0.99 ± 0.09 cm² 30 min after saline pre-treatment (difference not significant) (Fig. 1b). Variability in Amin was apparent at the 40- and 45-min time-points but the changes were not significant. Oxy-treated nostrils had baseline Amin of 0.80 ± 0.05 cm² that was increased by 46% to 1.17 ± 0.04 cm² ($P = 0.0001$).

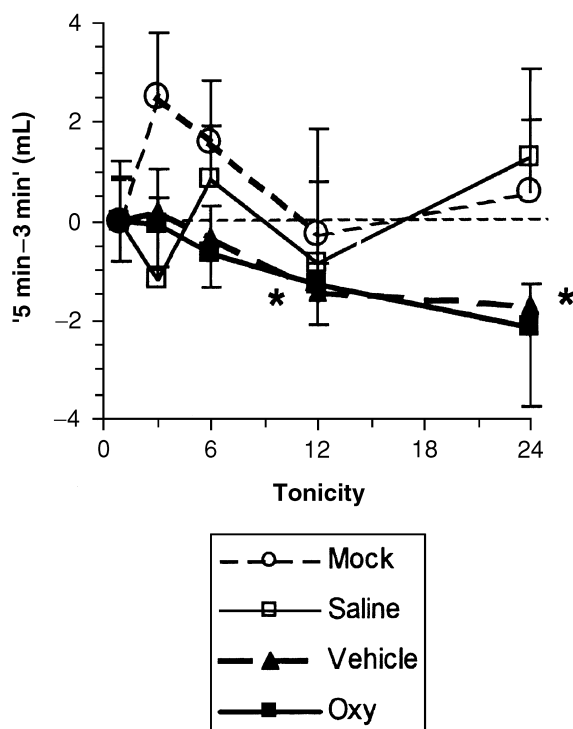


Fig. 4. Changes in V6 for the '5 min–3 min' period. Mock (open circles, dashed line) and serial saline (open squares, solid line) caused no significant changes from the initial provocation (homologous to 1 × NS). V6 decreased significantly after 12 × NS (* $P < 0.05$) and 24 × NS (** $P < 0.01$) following vehicle pre-treatment (solid triangles, dashed line). Although data for Oxy pre-treatment (solid squares, solid line) were equivalent, the wider variances precluded significance.

Effects of provocations on nasal airspace volume

There was a non-significant trend for '5 min–Pre' volumes to decrease in an HTS dose-dependent fashion after both saline and Oxy pre-treatments, with no changes after mock and serial saline provocations (data not shown). HTS had no effect in the first 3 min as '1 min–Pre', '3 min–1 min' and '3 min–Pre' values were not significantly altered (data not shown). As well, the 100 μ L volume of provocation solution had no significant effect as there were no changes at 3 × NS and 6 × NS and serial saline provocations.

In contrast, '5 min–3 min' volumes (Fig. 4) and Amin (data not shown) were reduced after HTS in dose-dependent fashion after saline and Oxy pre-treatment. V6 was significantly reduced by 12 × NS ($\Delta = 1.45 \pm 0.55$ mL, $P = 0.037$ vs. 1 × NS) and 24 × NS ($\Delta = 1.72 \pm 0.42$ mL, $P = 0.004$) after saline pre-treatment. Changes following Oxy pre-treatment were not statistically significant because of the wide variances in those data. Overall, 24 × NS caused reductions in V6 of similar magnitude (1–2 mL) after both Oxy and NS pre-treatments. The changes were much smaller than those induced by Oxy pre-treatment ($\Delta = 9.94 \pm 1.48$ mL). Therefore, Oxy pre-treatment did not alter the small HTS-induced changes in V6. Mock and serial saline challenges had no effect on '5 min–3 min' volumes or Amin.

The effects of blowing the nose were assessed by 'Blow–5 min' values (Fig. 5). There was a general but non-significant

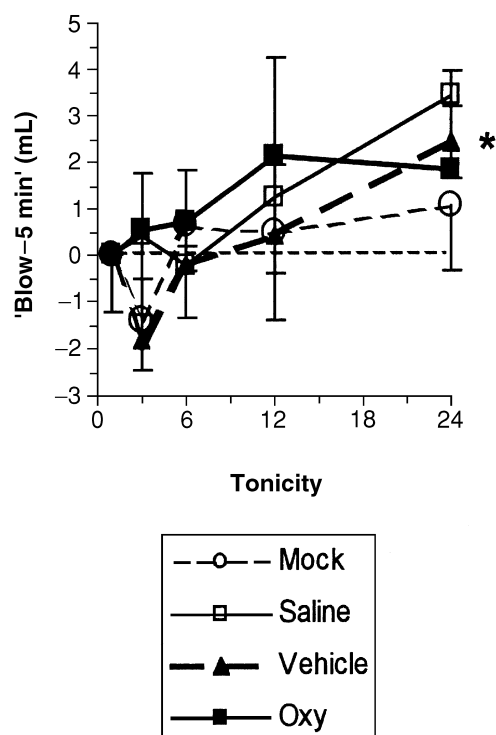


Fig. 5. Effect of blowing. There was a general trend for 'Blow–5 min' to increase with tonicity but the only significant change (* $P < 0.05$) was for vehicle pre-treatment (solid triangles, dashed line) at 24 × NS compared with 1 × NS.

trend for 'Blow–5 min' values to increase, but the only significant change was after saline pre-treatment and 24 × NS ($P < 0.05$). There were no significant differences between serial blowing, serial saline, Oxy and vehicle pre-treatments for HTS-related 'Blow–5 min' values.

Correlation between Acrh and lavage fluid weight

Weight was significantly and inversely correlated with '5 min–3 min' values for HTS-induced secretion after both vehicle ($r^2 = 0.88$) and Oxy ($r^2 = 0.94$) pre-treatments (Fig. 6). (Data for 1 × NS were subtracted from 3 ×, 6 ×, 12 × and 24 × NS results in this and the further analyses that follow.) Serial saline provocation results ($r^2 = 0.48$) were correlated only because of the final saline administration. There were no changes for mock provocations. 'Blow–5 min' was positively correlated with weight for serial saline ($r^2 = 0.83$), vehicle ($r^2 = 0.83$) and Oxy ($r^2 = 0.61$) pre-treatment (Fig. 7).

'Five minute–three minute' and 'Blow–5 min' volumes were negatively correlated for HTS provocations after Oxy ($r^2 = 0.77$) and vehicle ($r^2 = 0.73$) pre-treatments, but not mock or serial saline provocations (Fig. 8).

Effect of HTS on Amin

Changes in Amin paralleled those of V6. Amin was significantly reduced in the '5 min–Pre' period after 12 × NS (saline pre-treatment) and 24 × NS (Oxy pre-treatment) (data not shown). There were no changes in Amin in the first 3 min. However, 24 × NS reduced Amin in the '5 min–3 min' period by 0.067 ± 0.027 cm² after saline ($P = 0.035$) and

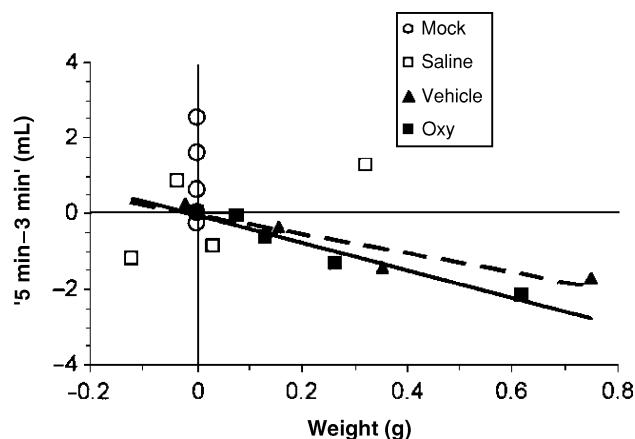


Fig. 6. Correlation of '5 min-3 min' volumes and net weights of collected lavage fluids. Weight was strongly correlated with the period of maximal secretion production ('5 min-3 min') following HTS. Results were nearly identical for vehicle (solid triangles, dashed line, $r^2 = 0.88$) and Oxy (solid squares, solid line, $r^2 = 0.94$) pre-treatments. The positive correlation for serial saline provocations (open squares, no line, $r^2 = 0.48$) was due only to changes in the final provocation of the protocol. The four points for each treatment group represent the incremental changes from $1 \times$ NS values for $3 \times$, $6 \times$, $12 \times$ and $24 \times$ NS results.

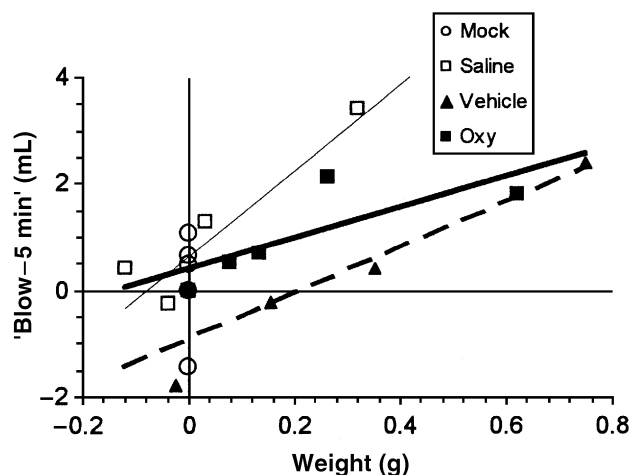


Fig. 7. Correlation of 'Blow-5 min' volumes and net weights. HTS induced significant correlations after vehicle (solid triangles, dashed line, $r^2 = 0.84$) and Oxy (solid squares, heavy line, $r^2 = 0.61$) pre-treatments. Serial saline provocations (open squares, light line, $r^2 = 0.83$) also showed this correlation because of the one outlying point. The four points for each treatment group represent the incremental changes from $1 \times$ NS values for $3 \times$, $6 \times$, $12 \times$ and $24 \times$ NS results.

$0.124 \pm 0.045 \text{ cm}^2$ after Oxy ($P = 0.02$) pre-treatments. A_{min} was increased during the 'Blow-5 min' period after $6 \times$ NS (saline pre-treatment, $P = 0.02$) and $12 \times$ NS (Oxy pre-treatment, $P = 0.005$), suggesting that secretions had been blown from the nasal valve region or that forceful exhalation had contracted facial muscles and dilated the alae nasae. There were no differences between Oxy and vehicle pre-treatments, indicating that any vasoconstrictive effect of Oxy did not affect the HTS-induced changes. The HTS-induced changes were much smaller in magnitude than the increase in A_{min} produced by Oxy pre-treatment ($\Delta = 0.481 \pm 0.071 \text{ cm}^2$).

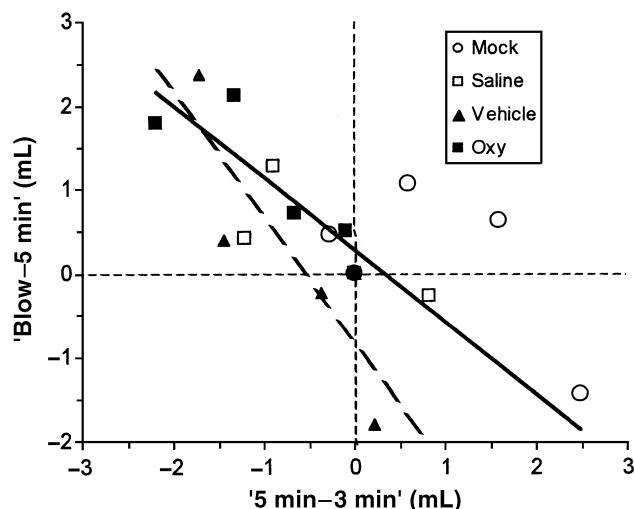


Fig. 8. Correlation of 'Blow-5 min' and '5 min-3 min' volumes. HTS induced highly correlated changes after vehicle (solid triangles, dashed line, $r^2 = 0.73$) and Oxy (solid squares, solid line, $r^2 = 0.77$) pre-treatments. Mock (open circles, $r^2 = 0.37$) and serial saline (open squares, $r^2 = 0.16$) did not have robust correlations. The four points for each treatment group represent the incremental changes from $1 \times$ NS values for $3 \times$, $6 \times$, $12 \times$ and $24 \times$ NS results.

Discussion

Acoustic rhinometry was used to assess nasal patency and estimate the degree of mucosal swelling following HTS provocations. The acoustic rhinometer emits sound waves at the nasal orifice that reflect from the lining of the nostril back to a detector [13,14]. Signal analysis provides measurements of the cross-sectional area as a function of distance from the detector [13-15]. The minimum cross-sectional area (A_{min}) of the anterior nasal valve may be reduced by mucosal swelling or increased by facial muscle contraction that dilates the alae nasae. The volume of the air within the nasal cavity is estimated by integration of the cross-sectional areas. As the volume of air is determined by the thickness of the nasal mucosa within the bony confines of the nose, the degree of mucosal swelling determines the nasal airspace volume. Rapid increases in airspace volume indicate thinning of the mucosal lining that is most likely to be due to vasoconstriction of the venous sinusoids.

Decreases in nasal airspace volume indicate swelling of the sinusoids, mucosal oedema due to increased plasma extravasation, or the presence of secretions in the airway lumen. These secretions may be due to vascular permeability with plasma exudation (as in rhinovirus infection [19]), or products exocytosed from submucosal glands and epithelial goblet cells (as after methacholine provocation [16] and in cholinergic rhinitis [20]). Because of the unpredictable changes in nasal patency due to the nasal cycle, it was necessary to perform bilateral nasal provocations, and to add results from the two nostrils together in order to detect the small but significant changes in nasal cavity geometry. Unilateral HTS provocations had no net effect on AcRh measurements in either the ipsilateral or contralateral nostrils [9].

Nasal patency is promoted by catecholamines released during exercise, increased sympathetic tone and α -adrenergic agonists

that activate $\alpha 1$ - and $\alpha 2$ -adrenergic receptor subtypes on sinusoidal vessels to induce vasoconstriction [13,17,18,21,22]. α -Agonists decrease superficial blood flow [13,17,23] but have no effect on the vascular permeability induced by histamine or allergen exposure [17,18]. Therefore, they may not affect the formation of mucosal oedema. Oxy significantly increased nasal patency, as has been shown for other α -adrenergic agonists [14,24–26]. V6 was increased by 9.9 mL from the basal 17 mL total volume (53% increase). Amin was increased by 43%. These changes represent a reversible component of nasal mucosal swelling and may serve as a surrogate measure of mucosal thickness.

HTS nasal provocation induced dose-dependent sensations of sharp pain, blocked airflow and rhinorrhea, and increased lavage fluid weights. Nasal airspace volume and Amin were reduced, with the changes limited to the fourth and fifth minutes of each provocation. This is consistent with previous work showing an increase in the weight of secretions after HTS and the presence of markers of glandular exocytosis as the predominant mucus constituents [1,4–9].

Markers of plasma extravasation were not altered, indicating that vascular leak from superficial fenestrated capillaries and post-capillary venules was not enhanced by HTS stimulation. This is similar to findings with capsaicin, the hot spicy essence of chilli peppers that also stimulates nociceptive nerves in human [27] and porcine [28] nasal mucosa. As a result, it is unlikely that significant mucosal oedema or plasma exudation could account for these HTS-induced effects. Correlations between weight, '5 min–3 min' and 'Blow–3 min' after HTS provocation suggest that glandular secretions generated during the fourth and fifth minutes were blown out. AcRh change after blowing could not detect clearance of these secretions as 'Blow–5 min' values for mock, serial saline and HTS provocation after vehicle and Oxy pre-treatments could not be segregated statistically.

In order to determine if HTS caused vasodilation, Oxy was used to pre-contract vessels. The key assumption was that if venous sinusoids were responsible for HTS-induced changes, then swelling should have been identified by a decrease in V6 and Amin after saline pre-treatment, but that there should have been no change after the maximal vasoconstriction induced by Oxy pre-treatment. The maximum change in V6 caused by HTS ($\Delta = 1.5$ mL) represented only 5% of the maximum airspace volume (26.3 mL after Oxy treatment). This 1.5 mL increase in volume represented only 15% of the 9.9 mL volume increase induced by Oxy. The absence of a statistically significant effect of Oxy pre-treatment on the HTS-induced changes in V6 make it unlikely that HTS could stimulate venous sinusoid vasodilation. Alternatively, small changes may have occurred that were below the limits of detection of acoustic rhinometry. Other methods may have detected these small magnitude changes. However, such small changes may not be of clinical relevance or affect nasal patency.

The timing of the AcRh changes after HTS offer a clue to the mechanism(s) regulating V6 and Amin. Pain was maximal in the first minute [4]. Oxy had no effect on maximum pain intensity but did reduce pain duration. As α -adrenergic agonists may activate inhibitory autoreceptors on nociceptive nerves, it is possible that Oxy decreased the 'slow pain' or 'second pain' mediated by non-myelinated C-fibres [29]. Oxy appears to have had no effect on the 'fast' or 'first pain' that is mediated by thinly myelinated A δ fibres and was identified by pain

intensity measurements. Subjective reports of blockage and drip and the decrease in V6 and Amin were maximal in the fourth and fifth minutes. These effects would be consistent with the hypothesis that HTS stimulated nociceptive nerves [1] leading to conveyance of a pain message to the cerebral cortex, release of neuropeptides in the nasal mucosa by the axon response mechanism [30,31] and stimulation of submucosal gland exocytosis followed by extrusion of mucus from glandular ducts [6–9].

We propose that in the normal human nose, irritants such as HTS stimulate neurogenic responses with glandular secretion as the predominant secretory event [4–9], and that vascular permeability and vasodilation are not significant components of neurogenic responses in normal human nasal mucosa [5–9]. Increases in nasal secretion production may cause relatively small changes in nasal airspace volumes, yet may significantly increase perceptions of rhinorrhea and nasal blockage. The dichotomy of increased perception of nasal obstruction or congestion with no change in objective measures of nasal airflow may be analogous to concepts of dyspnoea and measures of obstruction in chronic bronchitis, chronic obstructive pulmonary disease, hyperventilation and panic attacks.

This neural hypothesis may be extrapolated to bronchi and asthma. HTS stimulates 'non-specific bronchial hyper-reactivity' [2,3,32]. This 'non-specific' effect of HTS may be due to stimulation of nociceptive nerves that induce the sensation of dyspnoea, cough and the release of neuropeptides by the nociceptive nerve axon response mechanism that in turn may activate local smooth muscle contraction, glandular secretion or other bronchoconstrictive effects, either by direct activation of these end organs, or by depolarizing peribronchial parasympathetic post-ganglionic cells and causing acetylcholine-mediated contraction and exocytosis. Parallel bronchial provocation studies in asthmatic patients are required to assess this hypothesis.

These results offer new insights into the potential mechanisms of irritant responses in rhinitis. HTS did not induce vascular permeability or vasodilation that could be detected by AcRh in normal subjects. Superficial mucosal dilation analogous to the cutaneous flare response may be stimulated, but will require measurement of superficial blood flow for evaluation [23]. The prominent vascular leak induced by capsaicin in rodent airways was not found, suggesting that small animal models may not reliably predict human airway neural responses [10]. As HTS induced glandular secretion in the nose, it is possible that neural mechanisms regulate mucus secretion in both upper and lower airways. If confirmed in bronchial airways, then it is possible that the bronchoconstriction induced by HTS differs from the smooth muscle contraction that is thought to be induced by methacholine and histamine bronchial provocations.

In conclusion, HTS caused dose-dependent increases in sensations of pain, blockage and drip. There was a dose-related decrease in nasal volume ('5 min–3 min' data) but this effect was abrogated by blowing out nasal secretions (increased weights of returned lavage fluids). Oxy had no effect on pain intensity, discharge or the AcRh measurements induced by HTS. These findings indicate that constriction of vessels did not alter the consequences of HTS provocation. The excellent vasoconstrictor properties of Oxy were confirmed, yet this drug did not appear to significantly alter secretory or nociceptive mechanisms and had only minor effects on perceptions of nasal irritation.

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References

- Togias A, Lykens K, Kagey-Sobotka A et al. Studies on the relationships between sensitivity to cold, dry air, hyperosmolar solutions and histamine in the adult nose. *Am Rev Respir Dis* 1990; 141:1428–33.
- Anderson SD. Challenge tests to assess airway hyperresponsiveness and efficacy of drugs used in the treatment of asthma. *J Aerosol Med* 1996; 9:95–109.
- Anderson SD, Rodwell LT, Daviskas E et al. 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:S124–34.
- Ali M, Yuta A, Baraniuk JN. A comparison of nasal symptoms in normal and atopy subjects using a hypertonic saline (HTS) provocation. *J Respir Crit Care Med* 1997; 55:A978 (Abstract).
- Yuta A, Ali M, Baraniuk JN. Hypertonic saline (HTS): comparison of nasal secretion in normal and atopy subjects. *J Respir Crit Care Med* 1997; 155:A978 (Abstract).
- Ali M, Yuta A, Fang S, Baraniuk J. Neurogenic inflammation redefined. *J Allergy Clin Immunol* 1998; 101:S113 (Abstract).
- Ali M, Yuta A, Baraniuk J. Effect of oxymetazoline on hypertonic saline-induced neurogenic inflammation in the human nose. *Am J Respir Crit Care Med* 1998; 157:A720 (Abstract).
- Fang S-Y, Ali M, Yuta A, Baraniuk J. Hypertonic saline nasal provocation induces substance P release: airway neurogenic inflammation redefined. *Am J Respir Crit Care Med* 1998; 157:A487 (Abstract).
- Baraniuk JN, Ali M, Yuta A, Fang S-Y, Naranch K. Hypertonic saline nasal provocation stimulates nociceptive nerves, substance P release, and glandular mucous exocytosis in normal humans. *Am J Resp Crit Care Med* 1999; 160:655–62.
- McDonald DM. Neurogenic inflammation in the respiratory tract: actions of sensory nerve mediators on blood vessels and epithelium of the airway mucosa. *Am Rev Respir Dis* 1987; 136:S65–72.
- McDonald DM. Infections intensify neurogenic plasma extravasation in the airway mucosa. *Am Rev Respir Dis* 1992; 146:S40.
- Lewis T. *The Blood Vessels of the Human Skin and Their Responses*. London: Shaw and Sons, 1927.
- Roithmann R, Chapnik J, Zasmel N et al. Acoustic rhinometric assessment of the nasal valve. *Am J Rhinol* 1997; 11:379–85.
- Kesavanthan J, Swift DL, Bascom R. Nasal pressure–volume relationships determined with acoustic rhinometry. *J Appl Physiol* 1995; 79:547–53.
- Baraniuk JN, Ali M, Brody D et al. Glucocorticoids induce β_2 -adrenergic receptors in human nasal mucosa. *Am J Respir Crit Care Med* 1997; 155:704–10.
- Raphael GD, Druce HM, Baraniuk JN, Kaliner MA. Pathophysiology of rhinitis. I. Assessment of the sources of protein in methacholine-induced nasal secretions. *Am Rev Respir Dis* 1988; 138:413–20.
- Svensson C, Pipkorn U, Alkner U et al. Topical vasoconstrictor oxymetazoline does not affect histamine-induced mucosal exudation of plasma in human nasal airways. *Clin Exp Allergy* 1992; 22:411–6.
- Majchel AM, Baroody F, Kagey-Sobotka A et al. Effect of oxymetazoline on the early response to nasal challenge with antigen. *J Allergy Clin Immunol* 1993; 92:767–90.
- Igarashi Y, Skoner DP, Doyle WJ et al. Sources of nasal secretions during phases of experimental rhinovirus upper respiratory tract infections (URI). *J Allergy Clin Immunol* 1993; 92:722–31.
- Stjarne P, Lundblad L, Lundberg JM, Anggard A. Capsaicin and nicotine sensitive afferent neurones and nasal secretion in healthy human volunteers and in patients with vasomotor rhinitis. *Br J Pharmacol* 1989; 96:693–701.
- Stafford-Smith M, Baraniuk JN, Wilson K, Schwinn DA. α_2 -Adrenergic receptor subtypes in human nasal turbinate: expression of mRNA encoding specific α_2 -adrenergic receptor subtypes in nasal epithelium, duct, gland, and vascular cells. Assoc. of University of Anesthesiol. (Abstract), 1997.
- Richerson HB, Seeborn PN. Nasal airway response to exercise. *J Allergy* 1968; 41:269–84.
- Druce HM, Bonner RF, Patow C et al. Response of nasal blood flow to neurohormones as measured by laser-Doppler velocimetry. *J Appl Physiol Respir Environ Exercise Physiol* 1984; 57:1276–83.
- Roithmann R, Cole P, Chapnik J et al. Acoustic rhinometry, rhinomanometry and the sensation of nasal patency: a correlative study. *J Otolaryngol* 1994; 23:454–8.
- Fouke JM, Jackson AC. Acoustic rhinometry: effects of decongestants and posture on nasal patency. *J Lab Clin Med* 1992; 19:371–6.
- Corey JP, Kemper BJ, Nelson R, Gungor A. Evaluation of the nasal cavity by acoustic rhinometry in normal and allergic subjects. *Otolaryngol Head Neck Surg* 1997; 117 (1):22–8.
- Sanico AM, Atsuta S, Proud D, Togias A. Dose dependent effects of capsaicin nasal challenge: in vivo evidence of human airway neurogenic inflammation. *J Allergy Clin Immunol* 1997; 100:632–41.
- Stjarne P, Lacroix JS, Anggard A, Lundberg JM. Compartment analysis of vascular effects of neuropeptides and capsaicin in the pig nasal mucosa. *Acta Physiol Scand* 1991; 141:335–42.
- Dubner R. Neuropathic pain: new understanding leads to new treatments. *APS J* 1993; 2:8–11.
- Raphael GR, Baraniuk JN, Kaliner MA. How and why the nose runs. *J Allergy Clin Immunol* 1994; 87:457–67.
- Baraniuk JN. Neural control of human nasal secretion. *Pulm Pharmacol* 1991; 4:20–31.
- Bacci E, Cianchetti S, Paggiaro PL et al. Comparison between hypertonic and isotonic saline-induced sputum in the evaluation of airway inflammation in subjects with moderate asthma. *Clin Exp Allergy* 1996; 26:1395–400.