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Histamine Applied Topically to the Nasal Mucosa Increases the Trans-Mucosal Nitrous Oxide Exchange for the Middle-Ear

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Abstract

Objective—Determine if the middle-ear trans-mucosal Nitrous oxide (N₂O) exchange-rate is affected by nasal inflammation caused by topical application of histamine.

Methods—In a randomized, double-blind, crossover study, twenty adults were challenged intranasally with histamine (5 mg) and placebo on separate occasions. At each session, the subjects were fitted with a non-rebreathing mask and breathed room-air for 20 minutes, 50% N₂O:50% O₂ for 20 minutes and 100% O₂ for 10 minutes. Throughout, heart-rate, blood-pressure and blood O₂-saturation were monitored and bilateral middle-ear pressure was recorded by tympanometry every minute. The primary outcome measure was the slope of the middle-ear pressure-time function for the 50% N₂O:50% O₂ breathing period which is a measure of the trans-mucosal N₂O exchange-constant. The effects of Challenge Substance, Session and Period on the measured vital-signs and of Treatment, Session, Ear-Disease History and Test Ear on the pressure-time slopes were evaluated using repeated-measures ANOVAs.

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CONFLICT OF INTEREST STATEMENT

None of the listed authors have any real or apparent conflicts of interest with respect to the material presented in this manuscript.

IN MEMORIAM

William J. Doyle, Ph.D. (1.26.1952–10.21.2016)

Senior author William (Bill) J. Doyle Ph.D. passed away during the review of the current manuscript. Bill was a tenured Professor in the Department of Otolaryngology at the University of Pittsburgh and ran our research program at the Middle Ear Physiology Lab for 40 years. He was the principal investigator and co-investigator on dozens of NIH grants, authored and co-authored 289 peer reviewed articles (this will be #300), and served as an advisor or mentor to about 100 trainees at various levels.

Bill's extensive scientific work contributed to the better understanding of the roles of mucosal gas exchange and Eustachian tube function in middle ear pressure regulation. One of his last works, this manuscript was designed, analyzed and written under his supervision and is part of a triad of studies on modulation of middle ear gas exchange.

Bill is missed by his family, research partners and friends at the University of Pittsburgh and by the scientific community. His work will continue to be referenced and used as a guide for future research.

Results—The post-challenge total-symptom-score and the slope of the middle-ear pressure-time function were greater after histamine when compared to placebo challenge. Of the signs, only heart-rate was affected, responding to Challenge Substance and Study Period.

Conclusion—The trans-mucosal N₂O exchange-rate for the middle ear is increased during inflammation caused by nasal histamine exposure.

Keywords

Middle Ear; Nasal Challenge; Histamine; Blood Flow; nitrous oxide; gas exchange

INTRODUCTION

The middle ear (ME) cleft is a relatively fixed-volume, temperature stable gas pocket that is usually isolated from direct communication with the environment. The total pressure of that gas pocket follows the ideal gas law and, therefore, can be changed by changing pocket volume, temperature or the moles of contained gas. Regarding the latter, the ME can potentially exchange gas with each of four adjacent compartments: the local blood via gas transfer across the ME mucosa (MEM), the inner ear perilymph via transfers across the round window (RW), the environment via transfers across the tympanic membrane (TM) and the nasopharynx (NP) during active or passive openings of the Eustachian tube (ET). Because ME volume and temperature are relatively stable and gas transfer across the TM and RW are negligible^{1,2}, the active mechanisms that affect ME pressure, and consequently, the ME-ambient pressure balance are the passive gas-species exchanges between the ME and blood and the bolus gas flow through an open ET³.

Of the major physiological gases (O₂, CO₂, N₂ and H₂O), only N₂ has a measured trans-MEM pressure gradient large enough to drive a net gas transfer between the ME and mucosal blood. That transfer causes the loss of ME N₂ and the development of progressively more negative ambient-ME total pressure-gradients^{4,5}. ET openings at existing ambient-ME pressure deficits oppose that effect by replenishing the ME with gas of nasopharyngeal composition⁶ which partly or completely restores the ambient-ME pressure balance³. When the volume gas flow to (gas supply) and from (gas demand) the ME attributable to these two process is equal, the ambient-ME pressure gradient is maintained close to zero, a dynamic equilibrium that preserves MEM health and maximizes ME efficiency for hearing^{7,8}. This interactive, homeostatic mechanism of balanced volume gas transfers to and from the ME is referred to as ME pressure regulation (MEPR). Note that the efficiency of that mechanism can be downgraded if either trans-MEM gas diffusion is increased or if the frequency or efficiency of the ET opening is decreased³.

N₂ is an inert gas characterized by a relatively slow transMEM diffusion rate (*vis a vis* the other physiologic gases). Under physiologic conditions, the rate of N₂ exchange is directly dependent on the mucosal blood perfusion-rate such that increasing volume blood-flow will increase the N₂ exchange-rate and vice-versa⁹. Nitrous oxide, N₂O or laughing gas, shares this exchange property with N₂ and has been used in previous studies^{10–13} to determine the trans-MEM N₂ exchange properties. When administered properly, N₂O breathing is safe with a diffusion rate in tissue approximately 33 times faster than that of N₂, an advantageous

property that allows the N₂ diffusion rate to be estimated within a reasonably timed experimental period¹⁴.

Both epidemiological and physiological data suggest that the respiratory tract mucosa which extends from the ME to the ET, nose, sinuses and throughout the pulmonary tree, responds to local and systemic inflammatory stimuli as an integrated system, often referred to as the unified airway response. A variety of mechanisms are involved in driving this unified response, but the most important seem to be the “shared inflammation” that results from the continual communication among cellular and humoral components of mucosal immunity. Consequently, inflammation initiated in one discrete portion of the respiratory tract, the nasal mucosa for example, can be propagated and sustained through local-regional and systemic processes to the continuous ET and ME mucosa¹⁵.

Histamine, one of the most common and potent inflammatory mediators present in all body tissues, is released by mast cells and basophils in response to allergic and non-allergic stimuli¹⁶. In the nose, histamine release causes vasodilatation and nasal secretion leading to edema and mucosal congestion and has been used clinically for nasal provocation tests to investigate mucosal hyperactivity¹⁷. Intra-nasal challenge with histamine was shown to not only provoke specific symptoms and signs of local mucosal inflammation¹⁶, but also to cause inflammation of the ET mucosa expressed as a poorer ET opening efficiency^{18,19} and, in monkeys, to effect physiologic changes in the MEM expressed as an increased local blood flow²⁰ and an altered trans-MEM gas exchange-rate²¹.

In this experiment, we used histamine challenge of the nasal mucosa¹⁹ in the context of a previously described placebo controlled, double-blind, crossover protocol^{12,13} to test the hypothesis that the trans-MEM N₂O exchange-rate for humans is increased by histamine provoked nasal inflammation, an expression consistent with expectations under the unified airway model.

MATERIALS AND METHODS

Study Population

The study protocol was approved by the IRB at the University of Pittsburgh. Healthy adults, recruited by advertisement signed an informed-consent for study participation. Consented subjects provided medical and surgical histories and a comprehensive history for ME diseases, comorbidities and predisposing conditions. They had an Ear Nose and Throat examination with bilateral pneumatic otoscopy and tympanometry (Titan, Eden Prairie, MN) and women had a urine pregnancy test. Pregnant women and persons with an adverse reaction to gas-mixtures containing N₂O or fexofenadine, a history of chronic illness, had taken prescription medication within the previous month (except birth control), or had extant unilateral or bilateral otitis media, low tympanic membrane compliance or symptoms/signs of extant nasopharyngeal disease were excluded. Multiple allergic sensitivities (for example food, medication) and history of allergic rhinitis or asthma were also exclusion criteria.

Protocol

This was a randomized, double-blind, placebo-controlled, cross-over study requiring that participants complete paired N₂O breathing sessions at a minimum 1-week interval^{12,13}. Prior to subject entry, a pharmacist prepared the two challenge materials according to a randomization code and supplied them to the investigators as identical intranasal atomization devices (LMA MAD Nasal, Wolfe-Tory Medical Inc, Salt Lake City, UT) labeled only with session and subject numbers. The active substance consisted of histamine dihydrochloride 10mg/ml, sodium chloride 0.5%, sodium bicarbonate 0.275% and glycerin 50% (Positive skin test control - HollisterStier, Spokane, WA) and the placebo was a 50% glycerin-saline solution (Diluents, GREER, Lenoir, NC). Both were diluted by 50% in sodium chloride 0.9%, pH 7.0 and the histamine final mix concentration was 5mg/ml^{16,19}. Study personnel were blinded to the code until all recordings were double-entered into a computer data-file, reconciled and locked to change.

On presentation to a session, the subject provided an interval history for medication use, ME and upper respiratory diseases, had a brief ear, nose and throat examination and completed a 5-point symptom questionnaire rating each of the current symptoms of Runny Nose, Nasal Stuffiness, Sneezing, Cough, Itchy Eyes, Itchy Nose, Itchy Ears and Itchy Throat, from “none” (0) to “very severe” (5). The subject was comfortably seated on an examination chair and 1 ml of the blind-labeled substance was misted onto their nasal mucosa (0.5 ml per nostril) while they kept the palate closed by repeating the “K” sound. Approximately, 3 minutes after challenge, the subject re-scored the above listed symptoms. Then, the subject was fitted with the finger probe of a pulse oximeter (Massimo RDS1), the cuff of an automated blood-pressure monitor (Critikon Dynamap 1846SX) and a Rudolph Nasal & Mouth Breathing Silicone Face Mask (Model 8900, Kansas, USA) with a two-way non-rebreathing valve. Surface EMG electrodes were placed on the skin over the submental muscles to detect swallowing-related muscle activity and the chair was reclined to about 30 degrees to the horizontal. Three gas sources could be placed “on-line” to the mask, room air, 50%N₂O:50%O₂ and 100%O₂. Each breathing session consisted of a 20-minute acclimation period (Period 1, room-air breathing), a 20-minute experimental period (Period 2, 50%O₂:50% N₂O breathing) and a 10-minute recovery period (Period 3, 100% O₂ breathing). During Period 2, the subjects were asked to refrain from swallowing. Throughout, the submental EMG was continuously recorded, bilateral ME pressures were recorded by tympanometry at 1-minute intervals, blood O₂ saturation and heart-rate were monitored continuously and recorded every 5 minutes and blood-blood pressures were recorded every 5 minutes. At the end of Period 3, the subject was observed for 20 minutes, given a brief physical examination and dismissed if recovered from the N₂O breathing. At least 1-week later, the experiment was repeated using the crossover substance.

Data Analysis

The average blood O₂ saturation, heart-rate and systolic and diastolic blood-blood pressures for each subject and session for Periods 1 and 2 and the total of the 8 symptom scores for the pre and post-challenge periods were used as summary variables for analysis. The EMG was examined and times with patterns suggestive of a swallow identified. Each of 2 investigators (SC, JAE) examined the ME pressure-time functions at a 1-minute resolution and

independently identified linear segments uninterrupted by pressure change indicative of a functional ET opening. The slope of the identified linear segment was calculated by linear regression and used in the analysis as a measure of the standardized transMEM N₂O exchange-rate free of any confounding effects of ET openings.

The effect of challenge substance on the baseline-adjusted, post-challenge total-symptom-score was evaluated using a paired Student's t test. The effects of Challenge Substance (histamine, placebo), Test Session (1, 2) and Period (room air, N₂O) on the vital-signs were evaluated using repeated-measures ANOVAs operating separately on the blood O₂ saturation, heart-rate and the systolic and diastolic blood-pressure data. Similarly, the effects of Challenge Substance (histamine, placebo), Test Session (1, 2), Ear (right, left) and ME Disease History (positive, negative) on the ME pressure-time slope were evaluated for significance using a repeated-measures ANOVA. The NCSS 2007 statistical package, Kaysville, Utah, was used for statistical analyses and the format average±standard deviation is used consistently with an assigned *p* value for significance of 0.05.

RESULTS

Twenty enrolled subjects completed both challenge sessions as prescribed by the protocol. The average age of this population was 30.2±8.5 (range: 19.0 to 47.2) years. Ten subjects were male, 13 reported a self-assigned race as white, 10 reported a history of ME disease in childhood and none reported a positive history for allergy. Three other enrolled subjects completed the first but were lost to follow-up before the second challenge session. The incomplete data for those 3 subjects could not be included in the data analysis.

The average pre- and post-challenge total symptom scores were 0.8±1.4 and 14.7±11 for histamine and 0.7±1.5 and 11.2±8.7 for placebo, respectively. For both, the baseline-adjusted post-challenge score was significantly greater than 0 (Student's Paired t test, *p*<0.01) indicating that both substances provoked perceived symptoms. Using the same statistical test, the average histamine-placebo difference in the baseline-adjusted total-scores was 6.9±10.6, a value significantly greater than 0 and indicating a greater symptomatic response to histamine when compared to placebo (*p*<0.01).

Table I lists the averages and standard deviations of the four symptom/sign parameters for groups defined by a cross-listing of challenge substance (histamine vs. placebo) and period (room air vs N₂O mixture). A repeated-measures ANOVA that included "Individual", "Session", Challenge Substance", and "Period" as factors and all interactions was done separately for each parameter. That analysis showed that the factor, "Individual", explained a significant percentage of the variance in systolic and diastolic blood pressure and pulse (*p*<0.01 for all), but not blood O₂ saturation (oximetry), and that "Period" (*p*=0.015) explained a significant percentage of the variance in pulse. There was no significant effect of "Session", "Challenge Substance" or the interactions on any of the four parameters.

For the pressure-time slope, the repeated-measures ANOVA was run first with "Challenge Substance" (Histamine vs. Placebo), "Session Order" (1 vs. 2), "Ear" (Right vs. Left) and "OM History" entered as factors and considering all interactions and then repeated when

limiting the analysis to the four factors (See Table II). There were no significant effects on the pressure-time slope of any interaction, and only the effects of “Individual” and “Challenge Substance” on that variable were statistically significant. The average and standard deviation of the pressure-time slopes for the active and placebo treated sessions were: 7.3 ± 4.9 and 5.7 ± 3.3 daPa/min, respectively, and the average active-placebo difference was 1.5 ± 5.1 daPa/min. This shows that the N₂O exchange-rate was faster after the histamine when compared to the placebo challenge. This effect is shown for individual ears in the Figure which plots the slope recorded after the histamine challenge as a function of that recorded after the placebo challenge for the left and right ears of study subjects. Note that the majority of data points lay above the line of identity indicating a greater value after the histamine challenge.

DISCUSSION

In this experiment, we tested the generalizability of the unified airway response model¹⁵ to the continuous mucosa of the nose, ET and ME in humans within the context of a randomized, placebo-controlled, double-blind, study design. The unified airway response predicts that regional application of a noxious, inflammatory stimulus will provoke both local and distal mucosal inflammation. Here, topical application of histamine to the nasal mucosa was used as the regional stimulus^{16,18} and the trans-MEM N₂O exchange-rate, a direct measure of the MEM blood perfusion rate¹⁰, was measured as the distal inflammatory response^{12,13}. The confounding effect of ET openings on that response measure were reduced by reclining the subjects to decrease ET opening efficiency and instructing them to limit “swallowing” during the period of data collection, and eliminated by restricting the data analysis to time periods with no evidence of active or passive ET openings. Also, subjects were monitored for possible systemic effects of intranasal histamine challenge such as changes in Blood pressure, Pulse or O₂ saturation that could cause responses that mimic the patterns interpretable as increased MEM perfusion.

Analysis of the data documented a significantly greater increase in the total symptom score after the histamine when compared to the placebo challenge which evidences histamine-induced local inflammation, a significantly greater rate of trans-MEM N₂O exchange after the histamine challenge indicative of histamine-induced mucosal inflammation far distal to the site of exposure, but no effect of challenge substance on any of the measured vital signs. Moreover, the MEM response was not conditioned by the subject’s history of ME disease or by the order of application of the substances and was not different between the 2 ears of an individual. These results are consistent with our hypothesis that the trans-MEM N₂O exchange-rate for humans is increased by histamine provoked nasal inflammation and with the corollary that this is an expression of a unified airway response.

The results of previous studies provide additional support for the generalizability of the unified airway response to the continuous mucosa of the upper airway. For example, numerous studies in monkeys and humans showed that exposure of the nasal mucosa to histamine or to allergens in sensitized individuals caused the symptoms and signs of nasal inflammation/allergy and provoked a functional obstruction of the ET indicative of periluminal inflammation^{18,19,22–27}. In pilot studies done in monkeys, nasal challenge with

histamine was shown to cause a decreasing ME pressure and an increased MEM blood perfusion rate²⁰ and, as observed in the present study, to increase the transMEM N₂O exchange-rate²¹.

Interestingly, in the previous monkey studies, intranasal challenge with the pro-inflammatory chemical, PgD₂, caused a rapid increase in ME pressure that was attributed to a decreased ME volume consequent to PgD₂ induced MEM swelling²⁰. Because the post-challenge ME pressure trajectory is the main outcome variable for the present study, we considered the possibility that the pattern of ME pressure increase observed here signaled a change in MEM volume and not an increased MEM blood perfusion rate. However, we discounted that data interpretation because, 1) MEM swelling occurs quickly which would cause a rapid ME pressure response that is not consistent with the slow, progressive increase that characterized our data, 2) mathematical simulations of the experiment show that any reasonable change in MEM volume would have a very small effect on ME pressure, and 3) past experiments that used a similar design format and vasoactive agents to drive the response reported changes in the ME pressure trajectory similar to that observed in the present study^{12,13}.

The results for the present study hold relevance to the better understanding of the mechanisms of MEPR during inflammatory processes²⁸. While traditionally, MEPR efficiency was related directly to the efficiency of ET openings²⁹, as outlined in the Introduction, more recent “supply-demand” formulations of that mechanism show that MEPR efficiency also is indirectly related to the rate of trans-MEM N₂ exchange³. Because N₂O and N₂ are inert gases whose trans-MEM exchange-rate is perfusion-limited³, mechanisms that affect trans-MEM N₂O exchange are applicable to N₂ exchange²¹. Therefore, nasal pathologies that are associated with the local release of histamine such as nasal allergies³⁰ and, perhaps, viral upper respiratory tract infections³¹ can downgrade MEPR by both decreasing gas supply to the ME, downgrading ET opening efficiency, and increasing ME gas demand, increasing the rate of trans-MEM N₂ exchange. This mechanism effectively couples the expression of those nasal pathologies often described as comorbid conditions and OME.

These results and those for other studies done in our laboratory that used a similar format show that the trans-MEM N₂ exchange-rate and thus, the MEPR efficiency, can be pharmacologically modulated.

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Figure 1.

Scatterplot of the Period 2 ME Pressure-Time slopes recorded after the intra-nasal histamine challenge as a function of the paired slopes recorded after the placebo challenge for right and left ears of all subjects. The solid line is the line of identity for slopes recorded after the placebo challenge.

TABLE I

Cross-Tabulation of the Averages (Avg) and Standard Deviations (Std) of Systolic and Diastolic Blood Pressure, Pulse and Oximetry Data Recorded Over Period 1 (Room-Air Breathing), Period 2 (N₂O mixture Breathing) and the Period 1–2 Difference for the Histamine and Saline Challenge Sessions.

Parameter	Session	Period 1		Period 2		Period Difference	
		Avg	Std	Avg	Std	Avg	Std
Systolic	Histamine	114.6	9.9	115.9	11.3	1.3	6.9
	Placebo	114.9	10.9	116.7	13.5	1.7	4.3
Diastolic	Histamine	72.4	7.0	73.4	6.6	1.0	3.9
	Placebo	72.9	8.0	73.9	8.7	1.0	2.9
Pulse	Histamine	68.6	13.1	65.5	13.7	-3.2	5.8
	Placebo	65.5	11.5	62.8	9.9	-2.7	5.0
Oximetry	Histamine	98.9	1.4	99.8	0.4	0.9	1.3
	Placebo	99.2	0.8	98.7	5.0	-0.5	5.1

TABLE II

Result for of the Repeated-Measures ANOVA of the ME Pressure-Time Slopes listing the sources of variation and, for each source, the associated degrees of freedom (DF), sum of squares (SSQ), mean square (MSQ), Test Statistic (F-Ratio) and probability level (P-Value).

Source	DF	SSQ	MSQ	F-Ratio	P-Value
Person	18	600.5	33.4	2.6	0.003
Treatment	1	65.2	65.2	5.2	0.027
OM history	1	1.2	1.2	0.0	0.851
Ear	1	7.6	7.6	0.6	0.440
Session	1	16.8	16.8	1.3	0.254
Error	56	707.1	12.6		
Total (Adjusted)	78	1387.1			
Total	79				