Neurogenic inflammation evoked by hypertonic saline solution in chronic rhinitis

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Sheen-Yie Fang, M.D.
Professor
Dept. of Otolaryngology
Ntl. Cheng Kung University
138, Sheng Li Rd. Tainan, Taiwan
Abstract

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Objectives: When nasal mucosa is evoked by HTS, nasal congestion, edema and hypersecretion occurred. Whether these responses represent a direct effect on mucosal mast cells, epithelial cells that will release interleukins or a secondary response to sensory neuropeptides release are still unclear. Stimulation of nociceptive nerves with recruitment of parasympathetic reflexes is the postulated mechanism. This response needs further defined in the human nasal mucosa and it may be of greater magnitudes in subjects with established inflammation. To evaluate the sensory neuropeptides and parasympathetic neuropeptide in the neurogenic inflammatory reactions occurred in the hyperresponsiveness of chronic rhinitis

Methods: HTS nasal provocations (in vivo) in different concentration were performed on allergic rhinitis (AR), non-allergic rhinitis (NAR), sinusitis and normal subjects. Using RIA to quantify the sensory neuropeptides (SP, CGRP, NKA) and parasympathetic neuropeptide (VIP) in nasal secretion, we evaluate the nociceptive response and parasympathetic reflex. The components of vascular leakage and glandular exocytosis in each concentrated HTS provocation and subject groups were also investigated by ELISA.

Results: The sensory neuropeptides of nasal secretion will increase with a relation to the increasing concentrations of HTS. The parasympathetic neuropeptides were also detected to represent the parasympathetic reflexes involvement. We will prove the close relationship between hyperresponsiveness of chronic rhinitis and neurogenic inflammation in which the sensory neuropeptides play an important roles.
Introduction

When nasal mucosa is evoked by HTS, nasal congestion, edema and hypersecretion occurred. Whether these responses represent a direct effect on mucosal mast cells, epithelial cells that will release interleukins or a secondary response to sensory neuropeptides release are still unclear. Stimulation of nociceptive nerves with recruitment of parasympathetic reflexes is the postulated mechanism. This response needs further defined in the human nasal mucosa and it may be of greater magnitudes in subjects with established inflammation. Neurogenic inflammation evoked by non-specific irritants such as hypertonic saline (HTS), temperature, humidity play an important pathophysiological role of hyperresponsiveness in chronic rhinitis. Using HTS nasal provocations (in vivo), compare the glandular exocytosis and vascular permeability (secretion amount) in normal, allergic rhinitis (AR), non-allergic rhinitis (NAR), sinusitis and normal subjects. Using Radioimmunoassay (RIA), quantify the sensory neuropeptides (SP, CGRP, NKA) and parasympathetic neuropeptide (VIP) in nasal secretion from AR, NAR or sinusitis subjects to evaluate the nociceptive response and parasympathetic reflex. Using ELISA, quantify the components of vascular leakage and glandular exocytosis in each concentrated HTS and subject groups.

II. Materials and Methods

1. Patients:

The 30 patients who were divided into 3 groups: chronic rhinitis (allergic and nonallergic) (AR and NAR) and chronic sinusitis (CS). The diagnosis of chronic rhinitis and sinusitis were defined by typical symptoms and sinus CT scan findings. Allergy was diagnosed by typical allergic history, symptoms and positive skin tests. Normal control group enrolled 10 patients who have no nasal inflammatory diseases.

2. Nasal provocation tests (in vivo)

a. Subjects:
Ten patients from each group (total 40 patients) were studied after informed consent was obtained. Subjects were not studied within 3 weeks after an acute exacerbation. No medications were taken within 72 h of nasal challenge. The patients using steroid (topical or systemic), pregnancy or lactating will be excluded in this study.

b. Reagents:

- normal saline solution (0.15 mol/l) and hypertonic saline solution (4x and 16x)

c. Challenge Methods:

Subjects are seated and preexisting nasal secretions removed by spraying 10 sprays of normal saline (100 µg per spray) into both nostrils with a Beconase AQ bottle (Glaxo), and having the subject gently expell the secretions into a paper cup by expiration through the nose with the mouth closed. This process is repeated three times in 10 minutes. HTS provocation is performed, and 20 minutes later, 10 sprays of saline is applied to each nostril, and saline-induced lavage fluids collected. This simple modification of earlier methods is reproducible, simple, and well-tolerated. Sol and gel phases of lavage fluids are mixed gently, then frozen at -70°C for later analysis.

d. Radioimmunoassay (RIA)

The collected nasal lavage was weighted. The supernatants were aspirated, frozen, then lyophilized. The powdered extracts were reconstituted in a 0.04 M Na-phosphate buffer. Each sample was measured in duplicate using the RIA kits (Peninsula Laboratories) which included human VIP, SP, NKA and CGRP, [125I] peptides, polyclonal rabbit antisera to human VIP, NKA, SP and CGRP, goat anti-rabbit gamma globulin serum, and normal rabbit serum. Data for the neuropeptide concentrations were expressed as mean ±SE pg per mg protein in nasal lavage.

e. ELISA

Total protein. The nasal lavages were measured by the Lowry method using BSA as the standard.
**Albumin.** Human serum Albumin was measured by a competitive ELISA (Raphael et al. 1988).

**Lactoferrin (Lf) and Lysozyme (Ly) ELISA.** Lf and Ly was measured by a modified noncompetitive ELISA (White et al, 1987). 100 ul of rabbit anti-human Lf or Ly (Dako Corp.), diluted 1:1000 in 0.1 M carbonate buffer, pH 9.6 (coating buffer), was added to a Maxi-sorp 96 well microtiter plates and incubated at 37 degree C for 90 min. The wells were then blocked with 1% goat serum (Sigma) diluted in a buffer (PT) consisting of PBS, pH 7.4, and 0.05% Tween 80. Standards of Lf or Ly (Sigma) or sample (100ul) were then incubated at 37 degree C. For 90 min. The plates were washed and rabbit antihuman Lf or Ly-horseradish peroxidase conjugate, diluted 1:1000 in PT, was added to each well and again incubated at 37 degree C. For 90 min. The reaction was developed with an o-phenylenediamine dihydrochloride substrate and read at 490 nm. The assay range was between 1 and 200 ng/ml.

Alb percent (Alb %), Ly percent (Ly %), and Lf percent (Lf %). The Alb%, Ly%, and Lf% will be calculated by dividing the respective proteins by the total protein (TP) and multiplying by 100%.

**f. Statistics**

Data are expressed as the mean ±SEM. ANOVA tests will be used for statistical comparison. A p value of less than 0.05 was considered significant.
Results

The sensory neuropetides of nasal secretion will increase with a relation to the increasing concentrations of HTS. The parasympathetic neuropetides were also detected to represent the parasympathetic reflexes involvement. We prove the close relationship between hyperresponsiveness of chronic rhinitis and neurogenic inflammation in which the sensory neuropetides play an important roles. The following figures will show the nasal mucosa responses after HTS provocations. (These figures are in the Microsoft PowerPoint (file name: HTSARCHR2) (Attachment file 2)
Discussion

Chemical and mechanical irritation of the nasal mucosa, and electrical stimulation of the vagus nerves cause the release of neuropeptides from sensory nerves in the airway mucosa. These neuropeptides cause neutrophil adhesion (1), increase vascular permeability (2), and cause smooth muscle contraction (3), gland secretion (4), and cough (5). This constellation of effects is termed “neurogenic inflammation.”

Stimulation of nociceptive sensory nerves leads to the release of the tachykinins substance P (SP) and neurokinin A (NKA), as well as the release of calcitonin gene related peptide (CGRP) and probably gastrin releasing peptide (GRP). Sensory nerve stimulation and central reception of nociceptive nerve impulses in the brain stem and higher centers leads to the appreciation of sensations of itch, burning and congestion, and the initiation of important central reflexes such as sneeze, cough, and parasympathetic secretory reflexes (6). Neurogenic inflammation can induce the classic conditions of inflammation: dolor (pain due to central appreciation of nociceptive nerve stimulation), tumor (swelling due to vascular permeability), rubor (redness due to arterial dilation), and calor (sensation of heat due to arterial dilation and increased blood flow).

The many, diverse stimuli of sensory nerve may activate axon responses in the nasal mucosa. This "axon response" mechanism initiates and amplifies local mucosal vasodilation, vascular permeability, and glandular exocytosis, and may facilitate vascular wall leukocyte adhesion in the respiratory tract (1). However, in human nasal mucosa, axon responses need further investigation.

SP nasal provocation apparently has minimal effects in normal subjects, but induces albumin secretion and obstructs nasal airflow in allergic rhinitis subjects (7,8). Responses are greater in subjects with established inflammatory syndromes such as allergic rhinitis or asthma, suggesting that neurogenic inflammatory mechanisms play a role in these diseases or airway hyperresponsiveness.
Hyperresponsiveness is a characteristic of mucosal surface during inflammation. Hyperresponsiveness indicates an increased mucosal response to "non-specific" irritants such as histamine, methacholine, bradykinin, hypertonic saline solution, and other provocational agents. (9,10,11) Stimulation of nociceptive nerves with recruitment of parasympathetic reflexes is the postulated mechanism. This hyperresponsiveness plays a significant pathophysiological role in vasomotor rhinitis, although the cause of hyperreactivity remains unexplained. (12) Patients with vasomotor rhinitis have an enhanced reflexogenic secretory response to the irritant capsaicin. (13,14)

HTS aerosol challenge has been shown to be an effective bronchoconstrictor stimulus in asthma. (15) Suggested mechanisms include mediator release from primed mucosal mast cells and stimulation of neurogenic reflexes facilitated by the loss of epithelial integrity that occurs in asthma. (15) The parasympathetic system is an important neural bronchoconstrictor mechanism and along with peptidergic pathway plays an important role in the regulation of airway tone. It has been shown that hypertonic saline cause neurogenic inflammation in the eye in human beings and in the rat trachea. (16,17,18) Increased levels of histamine in nasal lavages of patients with allergic rhinitis after local, intranasal application of HTS, suggesting activation of mast cells, but whether this finding represents a direct effect on mast cells or a secondary response to sensory neuropeptide release is unclear. (19,20)
References


