Nasal sensory nerve populations responding to histamine and capsaicin

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Background: Inflammation of the nasal mucosa leads to sneezing, nasal itch, rhinorrhea, and nasal blockage. Many of these symptoms are likely the result of nasal trigeminal sensory nerve stimulation by inflammatory mediators. Nasal challenge with the C-fiber stimulant capsaicin causes a different set of symptoms than those evoked by histamine, suggesting that these 2 stimuli may activate separate subpopulations of nasal sensory nerves.

Objective: To investigate the trigeminal sensory nerves innervating the guinea pig nasal mucosa and to address specifically the hypothesis that histamine and capsaicin activate distinct subgroups of these nerves.

Methods: Guinea pig trigeminal neurons (retrogradely labeled from the nasal mucosa) were assessed for their responses to histamine and capsaicin by studying changes in the intracellular free calcium concentration, and assessed for substance P immunoreactivity.

Results: Only 60% of the nasal-specific trigeminal sensory neurons were found to be capsaicin-sensitive. Histamine stimulated only a subset (<40%) of these capsaicin-sensitive neurons. No nasal-specific capsaicin-insensitive neurons responded to histamine, although about 10% of trigeminal ganglion neurons per se responded to histamine but not capsaicin. Immunohistochemical analysis revealed that most (about 60%) of the sensory neurons innervating the nasal mucosa did not express the neuropeptide substance P, including nearly all large-diameter neurons, but also a significant number of small-diameter neurons (presumably C-fiber neurons).

Conclusion: Nasal neurons are not homogenous with respect to chemosensitivity or substance P content. It is likely that this heterogeneity in nasal afferent nerves underlies the differences in nasal responses to specific inflammatory mediators associated with the allergic reaction. (J Allergy Clin Immunol 2005;116:1282-8.)

Key words: *Trigeminal, nasal symptoms, sensory nerve, C-fiber, histamine, capsaicin, substance P*

Abbreviations used

DiI: DiC18(3)

Intracellular $[Ca^{2+}]_{free}$: Intracellular free calcium concentration

The nasal mucosa serves to warm and humidify inspired air while protecting the lungs from unwanted debris and pathogens. Key to this protection system is a complex neuronal system of both afferent and efferent pathways. The nasal mucosa is innervated by 2 distinct afferent sensory pathways: the olfactory nerve (cranial nerve I), which encodes information for the sensation of smell, and the trigeminal nerve (cranial nerve V), which encodes a wide variety of information on temperature, touch, airflow, occlusion, and chemosensitivity.[1-4]

Allergic inflammation of the nasal mucosa causes varying degrees of nasal blockage, sneezing, itch, and rhinorrhea.[5] In addition to the neuronally mediated sneeze and nasal itch, a substantial portion of rhinorrhea is the parasympathetic-mediated reflex hypersecretion,[6] and there is evidence supporting a role for afferent-released neuropeptides such as substance P contributing to nasal blockage.[7,8] Nasal sensory nerves are thus major transducers of symptoms associated with nasal inflammation. Activation of nasal sensory afferents may also have far-reaching effects on the lower airways and cardiovascular system.[9,10]

In human beings and animal models, nasal challenge with histamine mimics many of the symptoms of allergic nasal inflammation, including sneezing, nasal itch, rhinorrhea, and nasal blockage.[11-15] Like histamine, capsaicin can stimulate reflex hypersecretion, but the overall response to capsaicin can readily be distinguished from histamine in that it evokes intense burning pain, more than itch and sneeze.[16,17] This is the basis for the hypothesis that there are distinct histamine-sensitive fibers that encode itch and sneezing in the nose.[18] This hypothesis will remain untested, however, until more is known about the basic neurophysiology of trigeminal sensory innervation of the nose.

In the current study, experiments were designed to locate in the guinea pig trigeminal ganglion the cell bodies of afferents innervating the nasal mucosa, and assess these neurons for their immunoreactivity to substance P and their responses to histamine and capsaicin. Our data indicate that nasal afferents are localized in distinct regions of the trigeminal ganglion and that substance P is found only in a small subset of nasal afferents. In addition, we show that capsaicin is likely to stimulate a population of nerves not stimulated by histamine.

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METHODS

All experiments were approved by the Johns Hopkins Animal Care and Use Committee. Male Hartley guinea pigs (200-400 g; Hilltop Laboratory Animals, Inc, Scottsdale, Pa) were used.

Retrograde labeling of nasal trigeminal neurons

Nasal afferent neurons were retrogradely labeled by using DiC18(3) (DiI) solution (2%, in dimethyl sulfoxide). Under anesthesia (50 mg/kg ketamine and 2.5 mg/kg of xylazine; intraperitoneally), 30 μL DiI was instilled into the right nostril, and the guinea pigs were placed in a supine position leaning slightly to their right side. This was done to increase the exposure of the lateral wall of the nasal cavity, and thus the turbinates, to the DiI. The procedure was repeated the next day to label the left nostril. Small volumes of DiI frequently leaked from the contralateral nostril as a result of the hole in the caudoventral portion of the guinea pig septum. The external nasal area and facial fur was cleaned of any DiI by using ethanol. High levels of DiI labeling were detected throughout the nasal mucosal epithelium and submucosa of killed animals, but not in the tongue, eye, or facial skin. Very occasional DiI labeling was detected in the submucosa of the trachea, which is not innervated by the trigeminal ganglion.

Histology

Animals were killed 11 to 14 days after DiI labeling by 100% CO_2 asphyxiation, and the trigeminal ganglia were rapidly dissected and cleared of adhering connective tissue. The ganglia were fixed in paraformaldehyde (4%, in PBS) for 4 hours and then rinsed 3 times in PBS. Ganglia were cryoprotected overnight in 18% sucrose. Continuous serial sections (40 μm and 12 μm thick for topographical and immunohistochemical studies, respectively) of the trigeminal ganglia, starting at the caudal end, were thaw-mounted onto lysine-coated slides (40-μm slices were mounted consecutively; 12-μm sections were mounted on 4 different slides, such as the first slide had sections 1, 5, 9…, the second 2, 6, 10…, and so on; alternate slides were used for analysis). Slides were allowed to air-dry at room temperature in the dark. Slides prepared for immunohistochemistry (12-μm sections) were rinsed with water and PBS and incubated with goat serum (10%) diluted in PBS containing BSA (1%) at room temperature for 1 hour. Sections were then incubated with the primary antibody for staining substance P immunoreactivity (rat antisubstance P; 5 μg/mL; Chemicon, Temecula, Calif) diluted in PBS containing BSA (1%) for 24 hours at 4°C. After rinsing with PBS containing BSA (1%), the sections were covered with the secondary goat antirat Alexa Fluor 488-labeled antibody (20 μg/mL; Molecular Probes, Eugene, Ore) diluted in PBS containing BSA (1%) for 2 hours at room temperature. Slides prepared for both topographical and immunohistochemical studies were rinsed with PBS and with saline buffered with phosphate to pH 8.6, coverslipped, and viewed immediately. Sections were examined under epifluorescence (Olympus DX60 microscope, Melville, NY) by using appropriate filter combinations for DiI (excitation filter, 510-550 nm; barrier filter, 570-590 nm) and, when necessary, for Alexa Fluor 488 (excitation filter, 450-480 nm; barrier filter, 500-515 nm).

Cell dissociation

With the exception of the diphenhydramine experiments, all experiments studied afferent cell bodies harvested from guinea pigs that had been nasally labeled with DiI 11 to 14 days before sacrifice. After the animals were killed by 100% CO_2 asphyxiation, the trigeminal ganglia were rapidly dissected and cleared of adhering connective tissue. The rostral 3 mm of the medial part of each trigeminal was isolated from the rest of the ganglion (as determined by histology of nasally labeled trigeminal ganglion; Fig 1, *D*). The isolated tissue was incubated in the enzyme buffer (2 mg/mL collagenase type 1A and 2 mg/mL dispase II in 2 mL Ca^{2+}-free, Mg^{2+}-free HBSS) for 50 minutes at 37°C. Neurons were dissociated by trituration with 3 fire-polished glass Pasteur pipettes of decreasing tip pore size, then washed by centrifugation (3 times at 700*g* for 3 minutes) in L-15 medium containing 10% FBS. The cells were then resuspended in 100 to 150 μL L-15 medium containing 10% FBS. The cell suspension was transferred onto circular 25-mm glass coverslips (Bellco Glass Inc, Vineland, NJ) coated with poly-D-lysine (0.1 mg/mL), 25 μL per coverslip. After the suspended neurons had adhered to the coverslips for 2 hours, the neuron-attached coverslips were flooded with L-15 medium containing 10% FBS and used within 24 hours.

Intracellular calcium measurement

The intracellular calcium measurements were performed with dissociated trigeminal neurons irrespective of DiI labeling in a total of 19 animals. The coverslip was loaded with Fura 2 acetyoxymethyl ester (Fura-2 AM; 8 μmol/L) (Molecular Probes, Carlsbad, Calif) in L-15 media containing 20% FBS and incubated for 40 minutes at 37°C. The coverslip was placed in a custom-built chamber (bath volume of 600 μL) superfused with Locke solution (at 35°C) for 15 minutes before each experiment by an infusion pump (4 mL/min).

Changes in intracellular free calcium concentration (intracellular $[Ca^{2+}]_{free}$) were measured by digital microscopy (Universal; Carl Zeiss, Inc, Thornwood, NY) equipped with in-house equipment for ratiometric recording of single cells. For each experiment, a bright-field image and a fluorescent image (excitation filter, 510-550 nm; barrier filter, 570-590 nm) was taken of the field of cells under study. DiI-labeled cells were clearly identifiable. No DiI was observed in cells dissociated from ganglia isolated from animals that had not been nasally labeled. The field of cells was monitored by sequential dual excitation, 352 and 380 nm, and the analysis of the image ratios used methods previously described to calculate changes in intracellular $[Ca^{2+}]_{free}$.[19] The ratio images were acquired every 6 seconds. Superfused buffer was stopped 30 seconds before each drug application, when 300 μL buffer was then removed from the bath by using a taper made from Kimwipes tissue paper (Kimberly-Clark, Roswell, Ga). Drug, 300 μL, was then added gently to the bath during the period between 2 ratio image acquisitions. In each experiment, the cells on the coverslip were exposed to histamine (10 μmol/L) for 60 seconds (in the presence or absence of the H_1 antagonist diphenhydramine, 10 μmol/L). This was followed 90 seconds later with capsaicin (60 seconds, 0.5 μmol/L). Two minutes after capsaicin exposure, the cells were also exposed to KCl (30 seconds, 75 mmol/L) and then ionomycin (30 seconds, 0.5 μmol/L). KCl was used as an indicator of voltage sensitivity in cells that had a mean diameter of <15 μm and thus were not automatically assumed to be neurons. Ionomycin was used to obtain a maximal response. Between each stimulus, the cells were continuously washed with buffer. All drug concentrations were determined in preliminary experiments to produce near maximal responses.

In preliminary experiments, exposure to histamine (10 μmol/L) desensitized the neurons to further histamine challenge. Therefore, to test the sensitivity adequately of the neuronal response to H_1 antagonism, we compared the responses of diphenhydramine-treated and untreated neurons.

Drug preparations

Both capsaicin and ionomycin were diluted from 10 mmol/L stock solutions (dissolved in ethanol). Histamine and diphenhydramine were diluted from 100 mmol/L stock solutions (dissolved in distilled water). Ketamine, xylazine, histamine diphosphate, diphenhydramine, capsaicin, and ionomycin were purchased from Sigma-Aldrich