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

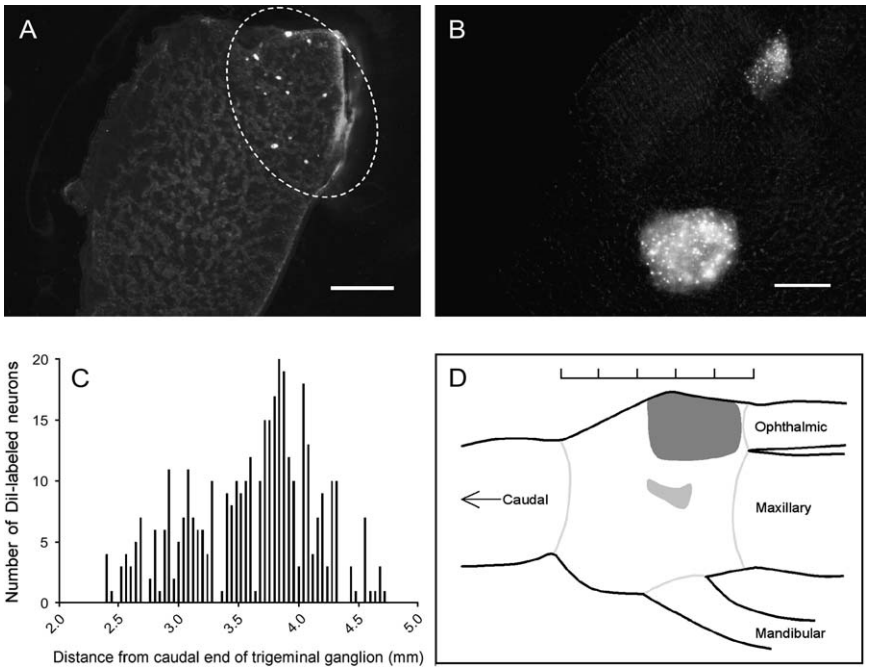

FIG 1. Retrograde labeling of trigeminal nasal neurons. **A**, Medial portion of ganglion indicated by the dotted area. Line represents 0.5 mm. **B**, Two nasally labeled neurons from *A*. Line represents 15 μm. **C**, Typical distribution of nasally labeled neurons throughout trigeminal ganglion. **D**, Schematic distribution of nasal neurons *(shaded areas)* in right trigeminal ganglion (dorsal aspect). Line represents 5 mm (1-mm intervals).

(St Louis, Mo). DiI and Fura-2 AM were purchased from Molecular Probes. L-15 and HBSS were purchased from Gibco Invitrogen Corp (Carlsbad, Calif).

Data analysis

For the analysis of Fura-2 AM–loaded cells, the measurement software converted ratiometric information to intracellular $[Ca^{2+}]_{free}$ by using a default set of Tsien parameters[20] particular to this instrumentation and a broad selection of cells. We did not specifically calibrate the relationship between ratiometric data and absolute calcium concentration, choosing instead to use the default parameters provided and relate all measurements to the peak ionomycin response in each viable cell. This effectively provided the needed cell-to-cell calibration for enumerating individual neuronal responses. The calcium concentration values given in the exclusion criteria below should not be considered absolute, but rather should be considered to be the calculated intracellular $[Ca^{2+}]_{free}$ on the basis of the assumptions mentioned. If a cell lacked a robust response to ionomycin (0.5 μmol/L) or had an averaged diameter (long and short axis) of less than 15 μm and produced a mean response to KCl (75 mmol/L) less than 40% peak ionomycin response, it was not included in the analysis. A robust response to ionomycin in this context was defined as a peak response within 90 seconds that had a calculated intracellular $[Ca^{2+}]_{free}$ greater than 400 nmol/L (mean peak ionomycin response was calculated to be 671 nmol/L with a SD of 315 nmol/L; n = 918). A specific neuron was considered to have responded to histamine (10 μmol/L) or capsaicin (0.5 μmol/L) if the mean response over the 60-second period of drug treatment (10 measurements) was greater than 2 SDs above the mean baseline response (60 seconds immediately before histamine treatment; n = 10 measurements). Each viable neuron was then allocated into 1 of 4 responder groups: capsaicin-sensitive/histamine-sensitive ($Cap^+/Hist^+$), capsaicin-sensitive/histamine-insensitive ($Cap^+/Hist^-$), capsaicin-insensitive/histamine-sensitive ($Cap^-/Hist^+$), and capsaicin-insensitive/histamine-insensitive ($Cap^-/Hist^-$). All cells, regardless of labeling, that passed the criteria were included in the analysis.

Fura-2 AM data were normalized to each neuron's maximum response to ionomycin (0.5 μmol/L). All data are expressed as means ± SEMs. Unpaired *t* tests and 2-way ANOVA were used for statistical analysis when appropriate, and a *P* value less than .05 was taken as a significant difference.

RESULTS

Location of nasal mucosal nerves in trigeminal ganglion

As expected, applying DiI to the nasal mucosa retrogradely labeled only a small subset of neurons in the trigeminal ganglion (Fig 1). The mean number of DiI-labeled neurons was 251 (±71) per ganglion (n = 3). Serial 40-μm sections, from caudal to rostral, indicated that DiI labeling was not randomly distributed; rather, DiI labeling occurred in a discrete area in the medial part of the ganglion, which is the source of the ophthalmic branch of the trigeminal nerve (Fig 1, *D*). Specifically, DiI-labeled nasal neurons were found in abundance throughout the rostral medial part of the trigeminal ganglion. In addition, scattered but distinct DiI-labeled neurons were observed laterally in a small area that is a source of the maxillary branch of the trigeminal nerve (Fig 1, *D*). DiI-labeled neurons in this area were almost exclusively found on the dorsal edges. Because of its high concentration of nasally labeled neurons, the rostral

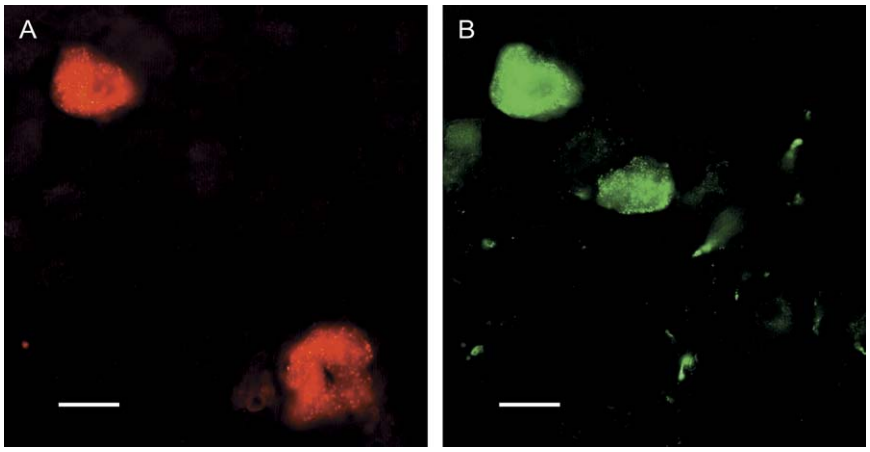

FIG 2. Photomicrographs of trigeminal ganglion. **A,** DiI-labeled nasal neurons. **B,** Immunohistochemical staining for substance P. Note that the smaller of the 2 DiI-labeled cells *(upper left corner)* also contained substance P. In addition, a non–DiI-labeled substance P–positive cell can be seen in the center of *B*. Line represents 25 μm.

medial part of the trigeminal ganglion was therefore chosen for subsequent studies of nasal afferent neurons.

Substance P in nasal mucosal cell bodies

DiI-labeled neurons in 12-μm slices of trigeminal ganglia were assessed for substance P content (Figs 2 and 3). Only a minority (37% ± 3%; n = 3 ganglia) of nasal-specific neurons were substance P–positive. The majority of large-diameter nasal neurons (≥35 μm, likely to be A-fibers[21]) were substance P–negative. Interestingly, a significant number of small diameter (≤25 μm, likely to be C-fibers[21]) nasal neurons were also found to be substance P–negative (Fig 3).

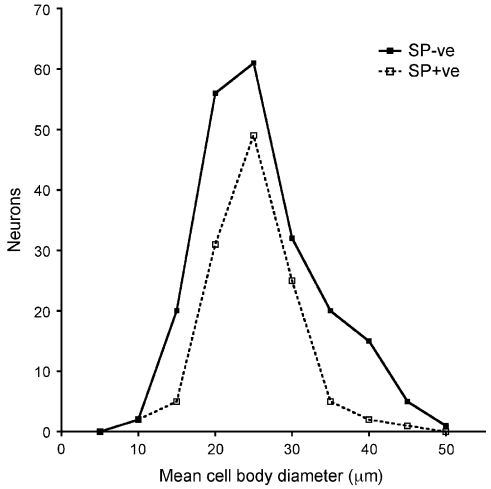

FIG 3. Immunohistochemical staining of substance P (SP) in nasally labeled neuron. Data are total cell counts recorded from 12-μm trigeminal slices harvested from 3 guinea pigs.

Response of trigeminal neurons to histamine and capsaicin

The increase in cytosolic calcium was used to monitor the direct response of neurons to histamine and capsaicin. We used a concentration of 10 μmol/L histamine because, in preliminary studies, we found that this provided a maximal response (ie, 100 μmol/L histamine did not produce different results). Fig 4 shows the time course of the change in intracellular $[Ca^{2+}]_{free}$ for the 48 nasal DiI-labeled neurons in response to 10 μmol/L histamine and 0.5 μmol/L capsaicin. The increase in intracellular $[Ca^{2+}]_{free}$ caused by histamine was smaller than the increase caused by capsaicin ($P < .01$, 2-way ANOVA). In addition, the histamine response appeared to be slightly slower in onset than the robust response to capsaicin.

The response to histamine and capsaicin revealed heterogeneity in the nasal trigeminal nerve population. Moreover, the nasal (DiI-labeled) neuronal population appeared to be different than the nonnasal (unlabeled) neuronal population with respect to histamine and capsaicin responsiveness (Fig 5). The majority (about 60%) of the unlabeled trigeminal neurons were unaffected by either capsaicin or histamine, whereas this was the case in only about 40% of nasal neurons ($P < .05$). About 20% of the nasal-specific population of neurons responded to both histamine and capsaicin, compared with about 6% of unlabeled neurons ($P < .05$). In addition, a greater percentage of nasally labeled neurons responded to capsaicin alone than unlabeled neurons (~40% vs 20%; $P < .05$). We found no nasally labeled neurons that responded to histamine but not to capsaicin. By contrast, about 10% of the unlabeled trigeminal neurons were found to be selectively stimulated by histamine. The average diameter of DiI-labeled capsaicin-sensitive neurons was significantly smaller than neurons that were capsaicin-insensitive (26.7 μm ± 1.0 μm vs 31.4 μm ± 2.1 μm; $P < .05$). Unlabeled neurons were significantly smaller than DiI-labeled neurons (20.8 μm ± 0.2 μm; $P < .05$).

Effect of H_1 antagonism on the responses to histamine and capsaicin

In 12 experiments, the H_1 antagonist diphenhydramine (10 μmol/L) significantly reduced the percentage of

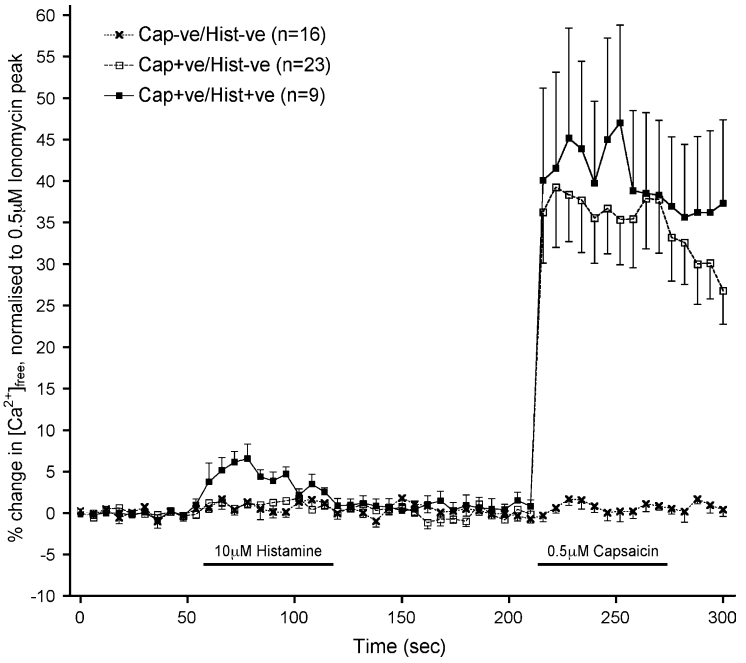

FIG 4. Effect of 10 μmol/L histamine (Hist) and 0.5 μmol/L capsaicin (Cap) on intracellular $[Ca^{2+}]_{free}$ in dissociated DiI-labeled nasal trigeminal neurons. Cell numbers for each group are shown in parentheses.

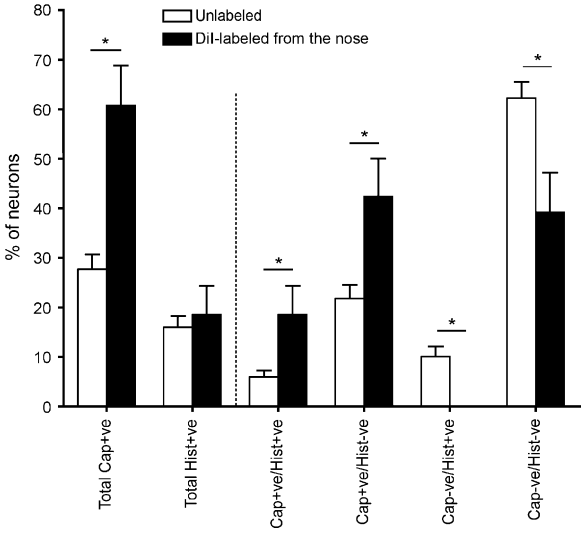

FIG 5. Responses of nasally labeled and unlabeled trigeminal neurons to 10 μmol/L histamine and 0.5 μmol/L capsaicin. Unlabeled data account for 59 experiments (643 neurons). Nasally labeled data account for 37 experiments (48 neurons). *Significant difference in the percentage of neurons in a particular responder group between unlabeled and nasally labeled trigeminal neurons ($P < .05$).

neurons that responded to 10 μmol/L histamine ($P < .01$; Table I). Diphenhydramine did not significantly affect the percentage of neurons responding to 0.5 μmol/L capsaicin ($P > .1$).

TABLE I. Effect of diphenhydramine (10 μmol/L) on the response to histamine and capsaicin†

	Vehicle	Diphenhydramine
Total histamine-sensitive	16.1% ± 2.2%	2.7% ± 2.2%*
Total capsaicin-sensitive	31.1% ± 3.1%	24.5% ± 2.8%

*Significant reduction in the percentage of responding neurons with the H_1 antagonist diphenhydramine ($P < .001$).
†Values are mean (± SEM) percentage of neurons that respond to either histamine (10 μmol/L) or capsaicin (0.5 μmol/L). Vehicle data account for 60 experiments (691 neurons). Diphenhydramine data account for 12 experiments (227 neurons).

DISCUSSION

The findings of the current study indicate that nasal trigeminal afferent nerves are homogeneous neither with respect to substance P content nor to their responses to capsaicin and histamine. Our data also demonstrated a clear topographical distribution of nasal afferent cell bodies within the trigeminal ganglion.

The majority of nasally labeled afferent cell bodies were concentrated in a region of the ganglion near the source of the ophthalmic branch of the trigeminal nerve. These afferents are likely part of the ethmoidal nerve,[22] a branch of the ophthalmic nerve that innervates the nasal mucosa.[4] We also found nasal afferent cell bodies located in a small area on the dorsal surface of the ganglion that is the source of the maxillary branch. These afferents are possibly part of the posterior nasal nerve or the nasal branch of the infraorbital nerve. Trigeminal topography of nasal afferents appears to be relatively conserved among small mammals, with all tracer studies reporting discrete labeled areas in the ophthalmic and/or ophthalmic/maxillary border areas: guinea pig,[23] rat,[24-28] and mouse.[29]

A perusal of published literature makes it clear that nasal challenge with histamine evokes sensations that are

different than those evoked by capsaicin.[16,17,30,31] Histamine does not cause pain, but causes itching and consistently induces sneezing. By contrast, capsaicin only occasionally and variably evokes sneezing, but consistently evokes sensations of intense burning pain. Both stimuli can evoke parasympathetic reflex hypersecretion. The differences in responses between these 2 sensory nerve stimulants may reflect differences in intensity of action potential discharge. On the other hand, our data support the hypothesis that the difference in sensations evoked by these 2 stimuli may also be a result of differences in the neuronal populations stimulated. Most of the neurons that responded to capsaicin did not respond to histamine. Or, put another way, the histamine-sensitive population of nasal afferents represented only a small subpopulation of capsaicin-sensitive nerves. It is tempting to speculate that stimulation of this small population of histamine/capsaicin-sensitive nerves leads to itching and sneezing on activation. Capsaicin, on the other hand, would activate both this histamine/capsaicin-sensitive population and the larger population of capsaicin-only sensitive nerves, resulting in sensations of burning pain. There is substantial evidence to suggest that painful stimuli inhibit the symptom of itch in the skin.[32-34] Thus, it is plausible that capsaicin, although activating both itch and pain fibers, causes mainly nasal pain as the sensation of nasal itch is actively inhibited.

In contrast with the nasal afferent population, the nonnasal trigeminal afferent population included capsaicin-insensitive neurons that responded to histamine. The location of the nerve terminals of these nonnasal trigeminal afferents is unknown, although it is recognized that the ophthalmic branch of the trigeminal innervates not only the nasal mucosa but also the cornea, iris, lacrimal gland, conjunctiva, and the skin of the eyelid, forehead, and nose. Histamine-sensitive capsaicin-insensitive afferent populations have been reported in rat dorsal root ganglia[35,36] and may represent a somatosensory itch-selective fiber.[37]

Histamine H_1 receptors are typically linked to G_q, and their activation leads to rapid increases in $[Ca^{2+}]_{free}$.[38] This allows for a convenient method to investigate whether a given neuron expresses functional H_1 receptors. It should be recognized, however, that increases in cytosolic calcium are not necessarily involved in action potential discharge at the nerve terminals. Nevertheless, our findings are, for the most part, consistent with observations made with extracellular recordings of action potentials in guinea pig nasal afferent nerves. That capsaicin activated 60% of nasal neurons is in agreement with guinea pig ethmoidal recordings in which capsaicin, instilled into the nasal cavity, activated 19 of 36 fibers.[3] Nasally instilled histamine has been shown to activate 9 of 15 single units recorded from the ethmoidal nerve of guinea pigs that had been pretreated with intranasal HCl.[39] In agreement with our data, all of these histamine-sensitive units were activated after nasal challenge with capsaicin. Our finding that histamine only activates nasal neurons that are also sensitive to capsaicin is also in agreement with *in vivo* studies showing that histamine-induced sneezing and parasympathetic-mediated reflex hypersecretion in guinea pigs are significantly reduced after capsaicin pretreatment.[15,40-42] We found that about 40% of the nasal afferent neurons were insensitive to both histamine and capsaicin. It is possible that this group represents nasal nonnociceptive A-fiber nerves given their significantly greater diameter than the capsaicin-sensitive afferents.

The H_1 receptor antagonist significantly reduced the number of dissociated neurons responding to histamine but not to capsaicin. Consistent with this, about 15% of guinea pig trigeminal afferents express H_1 receptor mRNA.[43] The sensitivity of the histamine response in trigeminal neurons to the H_1 antagonist diphenhydramine was not particularly surprising, because pharmacological studies in both human beings and guinea pigs have shown that H_1 antagonists reduce the sneezing and reflex hypersecretion caused by histamine and seasonal allergens.[11,14] The near abolition of the histamine-induced Ca^{2+} response by diphenhydramine does not preclude the presence of functional H_2 or H_3 receptors that do not have major effects on intracellular $[Ca^{2+}]_{free}$.

Substance P immunoreactivity was found in 37% of nasal afferent cell bodies in the trigeminal ganglia. Although the response characteristics of the substance P–positive population of neurons were not investigated here, it is likely that many (or all) of them are capsaicin-sensitive, because only larger dissociated neurons failed to respond to capsaicin, and large cell bodies rarely were substance P–positive. In addition, evidence from functional studies suggests that histamine stimulates a substance P–positive population of trigeminal neurons in guinea pigs.[23,44]

Even when large-diameter cell bodies (>30 μm) were discounted, a significant percentage of the remaining nasal afferent population was substance P–negative. This suggests that there may be a substantial C-fiber population of trigeminal nasal neurons that does not contain substance P. In the rat, 80% of neurons projecting to the nasal epithelium (labeled with rhodamine-labeled microspheres) were positive for substance P.[24] Perhaps our smaller percentage of substance P–positive neurons is a result of our DiI labeling method, which would label not just epithelial nerve terminals but also terminals innervating deeper into the nasal mucosa. Consistent with our finding in the guinea pig, 45% of ethmoidal-labeled feline trigeminal neurons were found to be substance P–positive.[45]

In summary, guinea pig nasally labeled afferent sensory nerves have been identified in the ophthalmic branch of the trigeminal nerve. The results provide the first clear evidence that these ophthalmic-derived afferents are not homogeneous with respect either to substance P content or to their responses to histamine or capsaicin. Histamine directly activates only a third of capsaicin-sensitive nasal neurons. Unlike the studied nonnasal neurons, there were no histamine-sensitive nasal neurons that failed to respond to capsaicin. Substance P staining was confined to nasal neurons with small diameters. More than half of small-diameter nasal neurons were not, however, positive for substance P immunoreactivity. All nasal symptoms are either totally or partially mediated by nasal sensory neurons; thus, it is likely that this heterogeneity of nasal

afferents underlies the differences in nasal responses to inflammatory mediators *in vivo* and may help to explain the complexity of nasal responses in allergy.

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REFERENCES

1. Sekizawa S, Tsubone H. Nasal mechanoreceptors in guinea pigs. Respir Physiol 1996;106:223-30.
2. Sekizawa S, Tsubone H, Kuwahara M, Sugano S. Nasal receptors responding to cold and l-menthol airflow in the guinea pig. Respir Physiol 1996;103:211-9.
3. Sekizawa SI, Tsubone H. Nasal receptors responding to noxious chemical irritants. Respir Physiol 1994;96:37-48.
4. Lucier GE, Egizii R. Characterization of cat nasal afferents and brain stem neurones receiving ethmoidal input. Exp Neurol 1989;103:83-9.
5. Skoner DP. Allergic rhinitis: Definition, epidemiology, pathophysiology, detection, and diagnosis. J Allergy Clin Immunol 2001;108:S2-8.
6. Widdicombe JG. Neuroregulation of the nose and bronchi. Clin Exp Allergy 1996;26(suppl 3):32-5.
7. Konno A, Numata T, Terada N, Hanazawa T, Nagata H, Motosugi H. Role of substance P in the vascular response of nasal mucosa in nasal allergy. Ann Otol Rhinol Laryngol 1996;105:648-53.
8. Piedimonte G, Hoffman JI, Husseini WK, Bertrand C, Snider RM, Desai MC, et al. Neurogenic vasodilation in the rat nasal mucosa involves neurokinin1 tachykinin receptors. J Pharmacol Exp Ther 1993;265:36-40.
9. Eccles R. Upper airway reflexes and involvement of the lower airway. In: Corren J, Togias AG, Bousquet J, editors. Upper and lower respiratory disease. New York: Marcel Dekker, Inc; 2003. p. 87-103.
10. Dutschmann M, Paton JF. Influence of nasotrigeminal afferents on medullary respiratory neurones and upper airway patency in the rat. Pflugers Arch 2002;444:227-35.
11. Taylor-Clark T, Foreman J. Histamine-mediated mechanisms in the human nasal airway. Curr Opin Pharmacol 2005;5:214-20.
12. Tiniakov RL, Tiniakova OP, McLeod RL, Hey JA, Yeates DB. Canine model of nasal congestion and allergic rhinitis. J Appl Physiol 2003; 94:1821-8.
13. Plevkova J, Kollarik M, Brozmanova M, Revallo M, Varechova S, Tatar M. Modulation of experimentally-induced cough by stimulation of nasal mucosa in cats and guinea pigs. Respir Physiol Neurobiol 2004;142: 225-35.
14. Fukuda S, Midoro K, Gyoten M, Kawano Y, Ashida Y, Nabe T, et al. Effects of TAK-427 on acute nasal symptoms and nasal obstruction in guinea pig model of experimental allergic rhinitis. Eur J Pharmacol 2003;476:239-47.
15. Imamura T, Kambara T. Substance P as a potent stimulator of sneeze responses in experimental allergic rhinitis of guinea pigs. Agents Actions 1992;37:245-9.
16. Plevkova J, Brozmanova M, Pecova R, Tatar M. Effects of intranasal capsaicin challenge on cough reflex in healthy human volunteers. J Physiol Pharmacol 2004;55(suppl 3):101-6.
17. Philip G, Baroody FM, Proud D, Naclerio RM, Togias AG. The human nasal response to capsaicin. J Allergy Clin Immunol 1994;94:1035-45.
18. Tai CF, Baraniuk JN. A tale of two neurons in the upper airways: pain versus itch. Curr Allergy Asthma Rep 2003;3:215-20.
19. MacGlashan D Jr. Single-cell analysis of Ca++ changes in human lung mast cells: graded vs. all-or-nothing elevations after IgE-mediated stimulation. J Cell Biol 1989;109:123-34.
20. Grynkiewicz G, Poenie M, Tsien RY. A new generation of Ca2+ indicators with greatly improved fluorescence properties. J Biol Chem 1985;260:3440-50.
21. Lawson SN, Perry MJ, Prabhakar E, McCarthy PW. Primary sensory neurones: neurofilament, neuropeptides, and conduction velocity. Brain Res Bull 1993;30:239-43.
22. Lucier GE, Egizii R. Central projections of the ethmoidal nerve of the cat as determined by the horseradish peroxidase tracer technique. J Comp Neurol 1986;247:123-32.
23. Tani E, Senba E, Kokumai S, Masuyama K, Ishikawa T, Tohyama M. Histamine application to the nasal mucosa induces release of calcitonin gene-related peptide and substance P from peripheral terminals of trigeminal ganglion: a morphological study in the guinea pig. Neurosci Lett 1990;112:1-6.
24. Hunter DD, Dey RD. Identification and neuropeptide content of trigeminal neurons innervating the rat nasal epithelium. Neuroscience 1998;83: 591-9.
25. Hunter DD, Satterfield BE, Huang J, Fedan JS, Dey RD. Toluene diisocyanate enhances substance P in sensory neurons innervating the nasal mucosa. Am J Respir Crit Care Med 2000;161:543-9.
26. Grunditz T, Uddman R, Sundler F. Origin and peptide content of nerve fibers in the nasal mucosa of rats. Anat Embryol (Berl) 1994;189:327-37.
27. Anton F, Peppel P. Central projections of trigeminal primary afferents innervating the nasal mucosa: a horseradish peroxidase study in the rat. Neuroscience 1991;41:617-28.
28. Schaefer ML, Bottger B, Silver WL, Finger TE. Trigeminal collaterals in the nasal epithelium and olfactory bulb: a potential route for direct modulation of olfactory information by trigeminal stimuli. J Comp Neurol 2002;444:221-6.
29. Dinh QT, Groneberg DA, Mingomataj E, Peiser C, Heppt W, Dinh S, et al. Expression of substance P and vanilloid receptor (VR1) in trigeminal sensory neurons projecting to the mouse nasal mucosa. Neuropeptides 2003;37:245-50.
30. Secher C, Kirkegaard J, Borum P, Maansson A, Osterhammel P, Mygind N. Significance of H1 and H2 receptors in the human nose: rationale for topical use of combined antihistamine preparations. J Allergy Clin Immunol 1982;70:211-8.
31. Kirkegaard J, Secher C, Borum P, Mygind N. Inhibition of histamine-induced nasal symptoms by the H1 antihistamine chlorpheniramine maleate: demonstration of topical effect. Br J Dis Chest 1983;77:113-22.
32. Toth-Kasa I, Jancso G, Bognar A, Husz S, Obal F Jr. Capsaicin prevents histamine-induced itching. Int J Clin Pharmacol Res 1986;6:163-9.
33. Schmelz M, Schmidt R, Weidner C, Hilliges M, Torebjork HE, Handwerker HO. Chemical response pattern of different classes of C-nociceptors to pruritogens and algogens. J Neurophysiol 2003;89:2441-8.
34. Wasner G, Schwarz K, Schattschneider J, Binder A, Jensen TS, Baron R. Interaction between histamine-induced itch and experimental muscle pain. Eur J Pain 2004;8:179-85.
35. Kim BM, Lee SH, Shim WS, Oh U. Histamine-induced Ca(2+) influx via the PLA(2)/lipoxygenase/TRPV1 pathway in rat sensory neurons. Neurosci Lett 2004;361:159-62.
36. Nicolson TA, Bevan S, Richards CD. Characterisation of the calcium responses to histamine in capsaicin-sensitive and capsaicin-insensitive sensory neurones. Neuroscience 2002;110:329-38.
37. Andrew D, Craig AD. Spinothalamic lamina I neurons selectively sensitive to histamine: a central neural pathway for itch. Nat Neurosci 2001;4: 72-7.
38. Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, et al. International Union of Pharmacology, XIII: classification of histamine receptors. Pharmacol Rev 1997;49:253-78.
39. Sekizawa S, Tsubone H, Kuwahara M, Sugano S. Does histamine stimulate trigeminal nasal afferents? Respir Physiol 1998;112:13-22.
40. Asakura K, Narita S, Kojima T, Saito H, Kataura A. Changes in nasal airway resistance and secretory response in the guinea pig after nasal challenge with capsaicin and histamine. Eur Arch Otorhinolaryngol 1994;251:224-8.
41. Konno A, Nagata H, Nomoto M, Motosugi H, Terada N. Role of capsaicin-sensitive trigeminal nerves in development of hyperreactive nasal symptoms in guinea pig model of nasal allergy. Ann Otol Rhinol Laryngol 1995;104:730-5.
42. Kokumai S, Imamura T, Masuyama K, Kambara T, Ishikawa T. Effect of capsaicin as a neuropeptide-releasing substance on sneezing reflex in a type I allergic animal model. Int Arch Allergy Immunol 1992;98:256-61.
43. Kashiba H, Fukui H, Morikawa Y, Senba E. Gene expression of histamine H1 receptor in guinea pig primary sensory neurons: a relationship between H1 receptor mRNA-expressing neurons and peptidergic neurons. Brain Res Mol Brain Res 1999;66:24-34.
44. Tani E, Shiosaka S, Sato M, Ishikawa T, Tohyama M. Histamine acts directly on calcitonin gene-related peptide- and substance P-containing trigeminal ganglion neurons as assessed by calcium influx and immunocytochemistry. Neurosci Lett 1990;115:171-6.
45. Lucier GE, Egizii R. Evidence for the presence of substance P in cat nasal receptor afferents. J Chem Neuroanat 1988;1:303-10.