TRPM5-Expressing Solitary Chemosensory Cells Respond to Odorous Irritants

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Lin W, Ogura T, Margolskee RF, Finger TE, Restrepo D. TRPM5-expressing solitary chemosensory cells respond to odorous irritants. J Neurophysiol 99: 1451–1460, 2008. First published December 26, 2007; doi:10.1152/jn.01195.2007. Inhaled airborne irritants elicit sensitive responses in trigeminal nerves innervating the nasal epithelium, leading to protective reflexes. The sensory mechanisms involved in the detection of odorous irritants are poorly understood. We identified a large population of solitary chemosensory cells expressing the transient receptor potential channel M5 (TRPM5) using transgenic mice where the promoter of TRPM5 drives the expression of green fluorescent protein (GFP). Most of these solitary chemosensory cells lie in the anterior nasal cavity. These GFP-labeled solitary chemosensory cells exhibited immunoreactivity for synaptobrevin-2, a vesicle-associated membrane protein important for synaptic transmission. Concomitantly, we found trigeminal nerve fibers apposed closely to the solitary chemosensory cells, indicating potential transmission of sensory information to trigeminal fibers. In addition, stimulation of the nasal cavity with high concentrations (0.5–5 mM) of a variety of odors elicited event-related potentials (ERPs) in areas rich in TRPM5-expressing solitary chemosensory cells. Furthermore, odorous chemicals and trigeminal stimuli induced changes in intracellular Ca²⁺ levels in isolated TRPM5-expressing solitary chemosensory cells in a concentration-dependent manner. Together, our data show that the TRPM5-expressing cells respond to a variety of chemicals at high exposure levels typical of irritants and are positioned in the nasal cavity appropriately to monitor inhaled air quality.

INTRODUCTION

Odorous chemicals are irritants at high concentrations, eliciting sensations such as burning, stinging, warmth, coolness, itching, or pain in the nasal cavity (Bryant and Silver 2000; Doty et al. 1978). Intranasal irritation signals poor air quality and potential health risks (Cain and Cometto-Muniz 1995). This sensation is transmitted by the trigeminal nerve innervating the nasal epithelium, as indicated by robust trigeminal responses to a large number of volatile odorous chemicals (Silver et al. 1986, 1991). It was assumed that these sensations arise from direct stimulation of intraepithelial free nerve endings. However, most of these endings are buried within the epithelial sheet making it difficult for stimuli to reach sensory endings quickly (Finger et al. 1990).

Recently, solitary chemosensory cells (also called solitary chemoreceptor cells) innervated by the trigeminal nerve have been identified in the anterior nasal cavity in rodents with an antibody against α-gustducin, a G protein α subunit found in taste receptor cells (Finger et al. 2003; Sbarbati and Osculati 2003; Zancanaro et al. 1999). Solitary chemosensory cells within the airways and gastrointestinal tract (Kaske et al. 2007) also were identified by an antibody directed against the transient receptor potential channel M5 (TRPM5), an ion channel critical in taste transduction (Perez et al. 2002; Zhang et al. 2003). Whether these cells are identical to the gustducin-expressing population was unclear. Interestingly, the α-gustducin–positive cells of the nasal epithelium express bitter-responsive type 2 taste receptors (T2Rs) similar to bitter-responsive taste cells. On the basis of these observations and the finding that bitter stimuli applied to the respiratory mucosa elicit trigeminal nerve responses, Finger et al. (2003) postulated that these α-gustducin–expressing cells are solitary chemosensory cells that mediate trigeminal responses to bitter-tasting substances.

Many inhaled irritants are odorous chemicals. The trigeminal nerve responds to high levels of most odors, but the mechanism underlying this response is unclear (Bryant and Silver 2000). Here we asked whether these or other nasal solitary chemosensory cells respond to odorous irritants. We used transgenic mice, in which the TRPM5 promoter drove the expression of green fluorescent protein (GFP; TRPM5-GFP) to identify the solitary chemosensory cells in this study. Members of the TRP family, such as the thermally sensitive TRPV1, TRPV4, TRPM8, and TRPA1, are expressed in neurons of trigeminal and dorsal root ganglia and interact with volatile compounds mediating sensations such as the coolness of mint and the hotness of chili peppers (Jordt et al. 2003; Silver et al. 2006; Voets et al. 2005). In addition, other TRP channels function as elements of G protein–coupled signaling cascades in chemosensory transduction. For example, TRPC2 is the transduction channel for chemical detection in the vomeronasal organ (Liman et al. 1999), and TRPM5 participates in taste transduction for sweet, bitter, and amino acid substances (Perez et al. 2002; Zhang et al. 2003), as well as semiochemical transduction in the olfactory epithelium (Lin et al. 2007).

Here we found that TRPM5 is expressed in a large population of solitary chemosensory cells, a subset of which coexpresses α-gustducin. Moreover, solitary chemosensory cells expressing TRPM5 respond to odors at high concentration with increases in intracellular Ca²⁺. This is the first demonstration at the cellular level that chemosensory cells in the respiratory epithelium respond to odorous irritants. Preliminary data were published in abstract forms (Lin et al. 2005; Ogura et al. 2006).

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METHODS

Animals

Adult C57BL/6 mice were purchased from the Jackson Laboratory (Bar Harbor, ME). TRPM5-GFP transgenic mice contain a TRPM5-GFP construct including 11 kb of mouse Trpm5 5′ flanking sequence, Trpm5 exon 1 (untranslated), intron 1 and the untranslated part of exon 2, and enhanced GFP (Clapp et al. 2006). Adult male cyclic nucleotide-gated channel A2 subunit (CNGA2) knockout mice were offspring produced by backcrossing the heterozygous CNGA2 knockout female mice (Brunet et al. 1996) (provided kindly by Dr. John Ngai) with wild-type FVB male mice. This resulted in knockout male offspring because the CNGA2 gene is X-linked and therefore hemizygous. Offspring were genotyped using PCR. All animal care and procedures were in compliance with the Animal Care and Use Committees of the University of Colorado Denver School of Medicine and University of Maryland, Baltimore County.

Immunocytochemistry

Tissue Preparation. Mice were anesthetized with ketamine/xylazine (100 and 20 μg/g body weight, respectively), perfused transcardially with 0.1 M phosphate buffer (PB) followed by a PB buffer containing 3% paraformaldehyde, 0.019 M t-lysin e monohydrochloride, and 0.23% sodium m-periodate (Lin et al. 1999). The nose was harvested and postfixed for 2 h. For direct visualization of GFP expression, the nose was split along the midline to expose the nasal cavity. Low-magnification pictures were taken using an Olympus dissecting and a Nikon E600 compound microscopes equipped with epi-fluorescence. High-magnification whole mounts were attached to slides using a small amount of OCT. Immunolabeling using tissue sections, bones surrounding the nose were removed after fixation, and tissues were transferred into PBS with 25% sucrose overnight and embedded with OCT. Fourteen-micrometer transverse sections were cut using a cryostat, mounted onto Superfrost plus slides (Fisher, Pittsburgh, PA), and stored in a –80°C freezer until used.

Cell Counting. For estimation of the number of TRPM5-expressing solitaire chemosensory cells in the respiratory epithelium, the TRPM5-GFP mice were fixed, and the nose was split along the midline. The respiratory epithelium from three hemi-noses were stripped, spread out, and mounted onto microscope slides with fluoromount-G (Fisher). Multiple images were taken at low magnification (×4) using an Olympus Fluoview confocal microscope. For immunolabeling using tissue sections, bones surrounding the nose were removed after fixation, and tissues were transferred into PBS with 25% sucrose overnight and embedded with OCT. Fourteen-micrometer transverse sections were cut using a cryostat, mounted onto Superfrost plus slides (Fisher, Pittsburgh, PA), and stored in a –80°C freezer until used.

Neurophysiology

Recordings of event-related potentials. The method was adapted from Lin et al. (2004). Mice were killed by CO2 inhalation, followed by cervical dislocation. The head was removed and split along the midline to expose anterior nasal respiratory epithelium. The half head was mounted on a recording chamber using the dental adhesive Impregum F (ESPE). Ringer solution was perfused continuously over the surface, and stimuli were delivered through a gravity-fed computer-controlled perfusion system with an approximate flow rate of 0.2 ml/s. Each stimulus was presented three times, and the largest response was used. Following stimulation, the epithelium was washed with saline for 2 min or until its response to Ringer solution was back to the basal level recorded at the beginning of the experiment. Event-related potentials (ERPs) are evoked local field potentials and were recorded using an Axopatch 200 B amplifier controlled by a PC computer with Axon software (Clampex 8, Axon Instruments, Union City, CA). Recording electrodes were filled with 0.9% agar made in Ringer solution with 1% neutral red or fast green and were placed on the apical surface of the anterior mucosa at similar locations marked in Fig. 1, where dense populations of TRPM5-positive cells were located. The reference Ag/AgCl electrode was connected to bath saline through an agar bridge. The recorded signals were low-pass filtered at 20 Hz, digitized at 500 Hz, and analyzed using the Axon software Clampfit.

Removal of the main olfactory epithelium. To determine whether olfactory sensory neurons contributed to the ERPs recorded from the reparatory epithelium, we removed the entire main olfactory epithelium and vomeronasal organ (VNO) in some animals. The nose of each mouse was split, and olfactory turbinate and the respiratory epithelium from the hemi-noses was reconstructed. The TRPM5-GFP mice were immunoreacted with antibodies raised against α-gustducin, phospholipase C β2 (PLCβ2) or the G protein γ subunit 13 (γ13), serial coronal sections from the anterior portion of the nose of TRPM5-GFP mice were immunoreacted with antibodies raised against α-gustducin, PLCβ2, or γ13. GFP-positive and immunoreactive cells were counted from fluorescence images taken using an Olympus epi-fluorescence microscope.

Immunolabeling. Sections or stripped epithelium were rinsed and incubated in blocking solution containing 2% normal donkey serum, 0.3% Triton X-100, and 1% bovine serum albumin in PBS for 1.5 h. Sections were incubated overnight or 72 h with primary antibodies against each of the following proteins: TRPM5, γ13 (1:250 or 1:500; Perez et al. 2002), PLCβ2 (1:200; sc-206, Santa Cruz Biotechnology, Santa Cruz, CA), calcitonin gene-related peptide (CGRP; 1:500; IHC6006, Peninsula Laboratories, San Carlos, CA), substance P (1:1,000; AB1977, Chemicon, Temecula, CA), α-gustducin (1:1,000; sc-395, Santa Cruz Biotechnology), or PGP9.5 (ubiquitin carboxyterminal hydrolase; 1:500; 7863–0504, Biogenesis, Sandown, NH). After incubation of the primary antibodies, sections were washed and reacted with donkey anti-rabbit secondary antibody (Alexa 555, Molecular Probes, Eugene, OR) for 1 h at room temperature. Sections were mounted on slides with Fluoromount-G. Controls for these experiments consisted of removing primary antibodies and using tissues from the TRPM5KO mice for the anti-TRPM5 antibody, which resulted in negative labeling. Images were taken using an Olympus Fluoview confocal microscope using the serial acquisition mode in cases involving dual fluorescent signals.

Ca2+ imaging

Fura-2 imaging was used to measure intracellular Ca2+ levels (Ogura et al. 1997, 2002). Solitary chemosensory cells isolation was adapted from the method of Rawson et al. (1997). Briefly, anterior respiratory epithelia of the TRPM5-GFP mice were removed from the nasal cavity after sacrifice, cut into small pieces, and placed in Ca2+- and Mg2+-free Ringer with 10–30 U/ml of papain (Worthington, Lakewood, NJ) and 2 mM cysteine for 30 min at room temperature.
FIG. 1. Transient receptor potential channel M5 (TRPM5)-expressing solitary chemosensory cells in the respiratory epithelium of the mouse nasal cavity. A: anterior nasal cavity viewed from a hemisected head from a TRPM5-green fluorescent protein (GFP) mouse, showing the GFP-positive solitary chemosensory cells densely populate the anterior part of the respiratory epithelium. A white circle indicates area where the event-related potential was recorded for Fig. 5. The anterior region of the main olfactory epithelium (MOE) is outlined in a white line. B: a magnified confocal image showing that the GFP-expressing cells are solitary, somewhat spindle-shaped. On average, there are 5,600 GFP-positive solitary chemosensory cells in the respiratory epithelium of a half nose. C: an image from a section through the respiratory epithelium showing that the apical processes (arrowhead) of a GFP-positive cell extends to the epithelial surface. D: a high-magnification confocal image from a whole mount of stripped epithelium showing several slender GFP-positive cells. Apical processes (arrowhead) and basal processes (arrow) emanate from the soma of these cells. E: anti-TRPM5 antibody (red) strongly labeled GFP-positive cells. The image was taken from a whole mount preparation of a stripped epithelium. Apical regions are marked by arrowheads. Scale bars: A, 1 mm; B, 50 μm, C–E, 10 μm.

The cells were detached by gentle pipetting. The cell suspension was transferred to an O-ring chamber on a coverslip precoated with poly-lysine (Sigma). After the cells settled, the solution was replaced with Ringer solution containing 2 μM fura-2/AM (Molecular Probes) for 20 min and washed with normal Ringer solution. Excitation wavelength was alternated between 350 and 380 nm using a filter wheel (Lambda 10-2, Sutter Instruments, Novato, CA) coupled to the output of a xenon lamp (model 770, Optiquip, Highland Mills, NY). The ratio of fluorescence intensity at excitation wavelengths of 350 and 380 nm was used to indicate the intracellular Ca\(^{2+}\) levels. Fluorescent images were obtained with a ×40 oil objective lens (NA 1.3) with a high-pass 525-nm emission filter (Chroma Technology, Rockingham, VA). Axon Imaging Workbench software version 2.2 (INDEC BioSystems, Santa Clara, CA) was used to capture images and to change the position of the filters. Image pairs were acquired every 2 s during responses and every 5–10 s during control and washout periods. To determine whether the extracellular Ca\(^{2+}\) influences the stimulus-induced Ca\(^{2+}\) response, we omitted the extracellular CaCl\(_2\) and repeated the same stimulation in the same cells after recording the control response in normal saline.

RESULTS

TRPM5 promoter–driven GFP expression in scattered cells throughout the respiratory epithelium lining the nasal cavity

We determined the location of TRPM5-expressing cells by monitoring GFP expression in the nasal cavity of TRPM5-GFP mice. We found expression of GFP in a subset of cells in the respiratory epithelium of the upper respiratory tract. Similar to solitary chemosensory cells reported by Finger et al. (2003) and Sbarbati and Osciulatti (2003), and the TRPM5-immunoreactive cells reported by Kaske et al. (2007), the GFP-expressing cells are scattered throughout the respiratory epithelium in the nose. In the nasal cavity, the GFP-expressing cells were concentrated anteriorly, near the entrance to the nasal cavity (Fig. 1, A and B). Figure 1, C and D, shows higher-magnification confocal images of GFP-expressing cells obtained from an epithelial section and stripped epithelium, respectively. These cells often were elongated and slanted across the respiratory epithelium with apical processes reaching the luminal surface (Fig. 1, C and D, arrowheads). In some of these cells, several spinous processes extended from the soma (Fig. 1D, arrow). None of these cells displayed axonal processes visible with the fluorescence microscope. The morphological characteristics of the GFP-expressing cells were similar to those α-gustducin–positive solitary chemosensory cells (Finger et al. 2003), but differed from the olfactory neurons in the main olfactory epithelium or in cells in the Grueneberg ganglion (Farbman 2000; Fuss et al. 2005; Koos and Fraser 2005). On average, there were 5,668.5 ± 1,029.6 (SE; n = 4) GFP-expressing solitary chemosensory cells in the respiratory epithelium on one side of the nasal cavity.

Immunoreactivity of TRPM5 in GFP-expressing solitary chemosensory cells

To determine whether the GFP-positive cells express TRPM5 protein, we performed immunolabeling using an anti-TRPM5 antibody in stripped epithelia containing GFP cells from the TRPM5-GFP mice. Strong immunoreactivity was observed in the GFP-positive cells. We picked at random 51
GFP-positive cells from three animals and imaged z-stacks of GFP fluorescence and fluorescence emitted by the secondary antibody in a laser scanning confocal microscope and in a regular fluorescence microscope. We found complete co-localization of GFP and TRPM5 immunoreactivity. A representative confocal image obtained from the whole mount preparation is shown in Fig. 1E. The specificity of the antibody against TRPM5 has been established by other investigators in previous studies (Clapp et al. 2006; Perez et al. 2002). No specific immunoreactivity was observed in control experiments where we omitted the primary antibody against TRPM5 (data not shown). We further confirmed the specificity of the TRPM5 antiserum by probing epithelia from TRPM5−/− mice. No staining of epithelial solitary chemosensory cells was observed in these knockout animals. These results showed that TRPM5 was expressed in the GFP-positive cells. In the experiments described later, we used GFP as a surrogate indicator of TRPM5 expression.

α-gustducin is expressed in a subset of GFP (TRPM5)-expressing cells

As indicated above, α-gustducin is expressed in a population of solitary chemosensory cells scattered throughout the nasal cavity (Finger et al. 2003; Zancanaro et al. 1999). In taste buds, a subset of TRPM5 expressing taste receptor cells coexpress α-gustducin (Perez et al. 2002). We tested whether TRPM5 and α-gustducin are coexpressed in some solitary chemosensory cells. Immunohistochemical experiments with an antibody against α-gustducin were performed on either coronal sections or whole mount preparations of the nasal epithelium from TRPM5-GFP mice. We found that immunoreactivity for α-gustducin was present in a subset of GFP-positive solitary chemosensory cells. The percentage of GFP cells expressing α-gustducin counted from both sections and whole mount epithelial strips was similar. On average for the entire nasal cavity, 14.6 ± 1.67% (n = 5) of GFP cells expressed α-gustducin [data pooled and averaged from the tissue sections (12.3%, n = 2) and whole mounts (16%, n = 3)]. A larger fraction of solitary chemosensory cells coexpressed GFP and α-gustducin in epithelial patches of the anterior respiratory areas (Fig. 2A, inset). Thus subpopulations of solitary chemosensory cells with different sensory properties may be present in the nasal respiratory epithelium.

Expression of signaling components of the PLC pathway

TRPM5 is a downstream signaling component of the PLC pathway in taste receptor cells (Liman 2007). To investigate whether other elements of the PLC pathway are present in the GFP-positive cells, we immunoreacted sections with antibodies against PLCβ2 and γ13, a G protein γ subunit associated with PLCβ2 activation in taste cells (Huang et al. 1999). We found that 62% of the GFP-positive cells expressed PLCβ2 (605 cells examined from 4 animals) and 73% of the GFP-positive cells expressed γ13 (903 cells examined from 4 animals). Immunoreactivity for PLCβ2 and γ13 in GFP-positive cells is shown in Fig. 2, B and C. At the level of light microscopy, we could not detect differences in morphology between GFP cells immunolabeled with anti-PLCβ2 and γ13 and GFP cells that were not immunoreactive. Although a higher fraction of GFP-positive cells expressed PLCβ2 and γ13 in the anterior portion of the nose, GFP-positive cells without immunoreactivity to these antibodies can be found throughout the respiratory mucosa. These results indicate that many but not all solitary chemosensory cells expressed PLCβ2 and γ13.

Close apposition of nerve processes to GFP-positive solitary chemosensory cells

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innervating the nose: PGP9.5, a pan neuronal marker to label all the nerve fibers (Finger et al. 2003), and substance P and CGRP to label trigeminal peptidergic fibers (data not shown). The fine processes of these cells and lack of GFP expression made it harder to visualize the cell boundary and determine whether they were innervated at light microscopic levels. It is likely that other potential solitary chemosensory cells, which do not express TRPM5, may be present in the nasal respiratory epithelium.

Expression of the vesicle-associated membrane protein synaptobrevin-2 in GFP-positive cells

The close association of the GFP-expressing solitary chemosensory cells and the trigeminal fibers suggests that solitary chemosensory cells may relay sensory information onto nerve fibers through synaptic transmission. We determined whether the GFP-positive solitary chemosensory cells express synaptobrevin-2, a vesicle-associated membrane protein critical for synaptic vesicle docking and exocytosis. Synaptobrevin-2 is associated with synaptic vesicles at synapses in the peripheral sensory systems, such as retina (von Kriegstein et al. 2003), cochlear hair cells (Safieddine and Wenthold 1999), and taste receptor cells (Yang et al. 2004). We found immunoreactivity for synaptobrevin-2 in 68 of 70 GFP-positive solitary chemosensory cells in 12 sections from two animals randomly examined (Fig. 4). Interestingly, positive immunolabeling for synaptobrevin-2 also was observed in nerve fibers running in the basal lamina of the respiratory epithelium and many fibers that contact the solitary chemosensory cells. Thus synaptobrevin-2 was present in both solitary chemosensory cells and the nerve fibers innervating them. In addition, we found 43 cells that showed immunoreactivity for synaptobrevin-2 but did not express GFP. Many of these non-GFP cells were spindle shape in the same cross-epithelial sections with fine basal processes reaching the basal lamina. We often found these cells in epithelial regions with relatively fewer synaptobrevin-2–positive nerve fibers (data not shown). The fine processes of these cells and lack of GFP expression made it harder to visualize the cell boundary and determine whether they were innervated at light microscopic levels. It is likely that other potential solitary chemosensory cells, which do not express TRPM5, may be present in the nasal respiratory epithelium.

Olororous chemicals evoked ERPs in the respiratory epithelium

The ERP recording is a common method used to investigate the local field potential changes evoked by irritants in the nasal respiratory mucosa (Hummel 2000; Rombaux et al. 2006). The ERP is also called the negative mucosal potential (NMP) by other investigators (Cain et al. 2006). Since many odorants at relatively high concentrations induce a sense of irritation (Bryant and Silver 2000), we examined whether strong odorants induced ERP responses in the areas densely populated by the TRPM5-expressing cells. ERPs were recorded at the location indicated by the area bounded by a red line in Fig. 1A, a region rich in GFP-expressing cells. At concentrations of ≈100 μM, the eight stimuli tested did not show apparent ERP responses (3 animals for each stimulus) except nicotine, which induced robust and repeatable responses (0.74 ± 0.34 mV; n = 4). At concentrations of 500 μM to 5 mM, all the stimuli tested (12 in total) induced repeatable ERP responses. The average amplitudes of the responses to individual stimuli ranged from 0.075 (kovanol) to 0.57 mV (ethyl propionate) at 5 mM. The largest amplitude traces to different stimuli selected from three to seven animals are shown in Fig. 5A. The averaged amplitudes for each stimulus (5 mM) are shown in Fig. 5B (n = 3–7). In addition, we monitored whether ERP depended on stimulus concentrations. Odorous chemicals at different concentrations were applied to different animals, and averaged ERP

FIG. 3. Innervation of the TRPM5-expressing cells. Trigeminal nerve fibers in the nasal cavity were visualized using antibodies against PGP9.5 and substance P. A: a confocal image showed PGP-immunoreactive nerve fibers closely apposing GFP-expressing cells in a whole mount preparation (arrows). B: in sections through the epithelium, PGP9.5-positive fibers can be observed wrapping the basal region of a GFP-expressing SCC and coursing along the cell body (arrowheads). C: substance P–positive fiber contacting a TRPM5-expressing cell. Multiple button-like swellings (arrowheads) were seen. Inset: an optical section showing that, in several regions, the GFP-labeled cell surrounds the nerve fibers indented into the soma (arrowheads). Scales: A, 10 μm; B, C, and inset, 5 μm.

FIG. 4. Expression of a vesicle-associated membrane protein synaptobrevin-2 in TRPM5-positive cells. A: immunoreactivity for synaptobrevin-2 was seen in the TRPM5-expressing cells (arrows), as well as presumed peptidergic nerve fibers that run along the basal lamina of epithelium and in close apposition to the TRPM5 cells. B: synaptobrevin-2 immunoreactivity (red) overlaid with images of TRPM5 cells (green). C: a magnified image showing synaptobrevin-2–reactive processes and TRPM5 cells. Apical processes are indicated by arrowheads. Scale: 10 μm.
To estimate whether the olfactory sensory neurons might contribute to the ERP responses, we recorded ERP from preparations in which we removed the entire olfactory epithelium, including the olfactory turbinate, septum, dorsal recess, VNO, and rostral end of the nasal cavity containing Grueneberg ganglion. The evoked ERP amplitudes (mV) under such condition were as follows: menthone, 0.12 ± 0.04; lilial, 0.25 ± 0.07; geraniol, 0.23 ± 0.04; 2-heptanone, 0.14 ± 0.05; pentyl acetate, 0.15 ± 0.03 (SE; n = 3; 5 mM concentration each). These ERP response amplitudes were not statistically different from the results shown in Fig. 5C (2-way ANOVA, P = 0.50, F = 0.46). In addition, we recorded ERP from cyclic nucleotide–gated channel subunit A2 (CNGA2) knockout mice. CNGA2 knockout abolishes most of the olfactory responses to common odor stimulation (Brunet et al. 1996; Lin et al. 2004). CNGA2 knockout mice displayed ERPs to odorous stimuli at the anterior respiratory areas similar to those of wild-type mice (data not shown). This result indicates that the respiratory epithelia, likely the TRPM5-expressing solitary chemosensory cells, mediated detection of high concentrations of odorous stimuli.

Odorous chemicals induce changes in intracellular $\text{Ca}^{2+}$ levels of the solitary chemosensory cells

To determine whether the GFP (TRPM5)-positive solitary chemosensory cells were responsive to volatile irritants, we monitored intracellular $\text{Ca}^{2+}$ levels in freshly isolated GFP-positive cells loaded with the fluorescent $\text{Ca}^{2+}$ indicator Fura-2. A variety of odorous chemicals at 0.5 mM induced an increase in the intracellular $\text{Ca}^{2+}$. Figure 6A shows representative $\text{Ca}^{2+}$ responses in the GFP-expressing cells. A repeated response to lilial in the same cells was also shown. The amplitudes of $\text{Ca}^{2+}$ responses to odorous stimuli in the GFP-positive cells were concentration-dependent; higher concentrations of stimuli evoked larger $\text{Ca}^{2+}$ responses (Fig. 6B). At concentrations of 20 μM, there were little or no responses to either lilial or geraniol, but at 500 μM, both compounds produced robust responses. The responsiveness of each cell tested is shown in Fig. 7. Each cell responded to at least one stimulus. Many cells responded to high concentrations of lilial (88% of 17 cells), geraniol (61% of 18 cells), and citral (50% of 18 cells). Only 1 of 16 cells responded to urinary components, such as 2, 5-dimethylpyrazine (DMP) and 2-heptanone. Interestingly, most cells responded to multiple, but not all stimuli tested, suggesting selectivity in these GFP-positive
cells. Taken together, our data provide the first direct evidence that the TRPM5-expressing solitary chemosensory cells respond to high concentrations of diverse volatiles.

Since TRPM5 is the downstream effector of the PLC signaling pathway, we tested involvement of the PLC pathway in the responses to volatile substances by using the PLC inhibitor U73122. In the presence of U73122 (5 μM), the response to lilial was reduced significantly (Fig. 6C; n = 5). We had attempted to wash out the U73122 effect. Three of five cells tested were partially recoverable after 10 min of washing with regular saline. Additionally, we tested whether the intracellular Ca\(^{2+}\) increase was caused by a Ca\(^{2+}\) influx from the extracellular medium or Ca\(^{2+}\) release from intracellular stores. We found that the amplitudes of Ca\(^{2+}\) responses to geranial (500 μM) decreased when cells were bathed in zero Ca\(^{2+}\) extracellular solution. The average amplitude of Ca\(^{2+}\) response was 62 ± 14% of the control responses obtained in normal saline in the same cells (n = 5; paired t-test, P = 0.14). This result indicated that both Ca\(^{2+}\) influx and Ca\(^{2+}\) release from the intracellular stores may contribute to the irritant-induced Ca\(^{2+}\) responses in the solitary chemosensory cells. Our data suggest that the PLC pathway and TRPM5 may be involved in detection of volatile chemicals in the GFP-expressing solitary chemosensory cells.

**DISCUSSION**

High concentrations of nearly any odorant compound produce a sensation of irritation in the nasal cavity. Previous investigators have assumed that the volatile compounds are detected directly by the free endings of the trigeminal nerve, but the recent discovery of solitary chemosensory cells in the nasal cavity offers a possible alternative for detection of such substances. Previous study on solitary chemosensory cells has shown that some of α-gustducin–expressing solitary chemosensory cells express members of the T2R family of taste receptors (for bitter substances) and trigeminal fibers respond to bitter-tasting ligands applied in solution to the nasal cavity (Finger et al. 2003). Here we sought to investigate the population of TRPM5-expressing solitary chemosensory cells and their chemical responsiveness to volatile irritants. We used both transgenic animals and immunolabeling to identify cells that express TRPM5, an ion channel that is present in taste receptor cells and belongs to the TRP family of ion channels with members known for their role in chemosensory transduction. We found that TRPM5 is expressed in a large population of cells in the nasal respiratory epithelia morphologically similar to those expressing α-gustducin. Furthermore, we demonstrated that high levels of various odorants induced local ERPs and changed intracellular Ca\(^{2+}\) levels of isolated TRPM5-expressing solitary chemosensory cells. This is the first direct demonstration that TRPM5-expressing solitary chemosensory cells in the nasal cavity respond to odorous chemical irritants.

**Relation with the α-gustducin–expressing solitary chemosensory cells**

The TRPM5-expressing cells share similar morphology with α-gustducin–expressing solitary chemosensory cells described previously (Finger et al. 2003). In the nasal cavity, as in taste buds (Perez et al. 2002), only a subset of TRPM5-expressing cells expresses α-gustducin. In rats, the maximum density of α-gustducin–expressing nasal solitary chemosensory cells was ~300 cells/mm\(^2\), and the total number was estimated at 1,000 in each half of the nose (Finger et al. 2003). We found significantly more TRPM5-positive solitary chemosensory cells in the nasal cavity of mice, roughly 11,200 per nose (5,600 in each nostril). Only ~15% of the TRPM5-positive
solitary chemosensory cells also express α-gustducin in mice, so there seemed to be roughly the same number of gustducin-positive solitary chemosensory cells in the two species.

TRPM5-positive taste receptor cells express members of the PLC transduction cascade and some also express gustducin (and presumably T2R taste receptors). We found that immunoreactivity for both PLCβ2 and γ13 was present in most of the solitary chemosensory cells. It is not known whether the GFP-positive cells that are not immunoreactive for PLCβ2 and γ13 represent solitary chemosensory cells at different developmental stages or different cell types. The nasal solitary chemosensory cells have a limited life span, turning over about every 2 mo (Gulbransen and Finger 2005). If immature solitary chemosensory cells express TRPM5-driven GFP before PLCβ2, gustducin, or γ13, this would account for the presence of solitary chemosensory cells that exhibit GFP fluorescence but are nonimmunoreactive for these other signaling molecules. Conversely, some solitary chemosensory cells may use other transduction cascades to trigger the TRPM5 channel.

In any event, we found that a PLC pathway blocker suppresses responses of solitary chemosensory cells to odorous chemicals, suggesting that at least some of the TRPM5-expressing solitary chemosensory cells use a PLC-mediated pathway in detecting odorous irritants. However, further experiments are required to determine whether TRPM5 itself is an obligatory element in the transduction of nasal irritants.

**Differences between trigeminal-mediated and olfactory-mediated detection of odorous chemicals**

Both the olfactory and trigeminal systems detect volatile chemicals. However, these two systems differ fundamentally. First, the olfactory system in rodents expresses ~1,000 odor receptors, allowing detection and discrimination of a vast array of odorants with exquisite sensitivity. Odor detection by the olfactory system elicits a sense of smell for individual odorants, whereas detection by the trigeminal system provides a sense of irritation, ranging from tingling to pain with limited discrete categories (Bryant and Silver 2000). In contrast to the fine discriminative abilities for the sense of smell, quality discrimination for trigeminal irritants is quite poor. Second, there is a significant difference in the detection thresholds in the two systems. Thresholds in the olfactory system are in most cases several orders of magnitude lower than the thresholds estimated for irritation effects of the upper airways (Cometto-Muniz and Cain 1990, 1994, 1995; Cometto-Muniz et al. 2004; Doty et al. 2004). In our previous studies, we examined odor-evoked changes in local field potential in the main olfac-

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**Fig. 7.** Responding profile of GFP-expressing solitary chemosensory cells. GFP-expressing cells respond to volatile stimuli. A total of 18 responding cells were stimulated with 4 or more chemicals. ●, cell responded; ○, cell did not respond.
try epithelium using electro-olfactograms (EOG) recording (Lin et al. 2004), a method similar to ERP recording. Both lilial and geranial (1 μM) induced changes in EOG in the olfactory epithelia (0.44 ± 0.06 mV, n = 7; 0.45 ± 0.07 mV, n = 10, respectively, unpublished data). In contrast, we were never able to record any responses—either ERP or Ca^2+ imaging of TRPM5-expressing cells—for these odorous stimuli at such low concentrations.

Finally, a multitude of G protein–coupled odor receptors (~1,000 in mice) trigger the cAMP signaling pathway mediating olfactory function in olfactory sensory neurons that transfer the signal directly to the olfactory bulb through their axons. In contrast, here we showed that the trigeminally mediated chemical sensation likely involves detection and neurotransmitter-mediated transmission of information to nerve endings. In addition, although TRPM5 has not been demonstrated in free nerve endings, studies on gene targeted mice strongly suggest that some chemicals interact directly with ion channels in the nerve endings (Jordt et al. 2003). Trigeminal sensory neurons receiving input from the nasal cavity show chemosensory properties and respond to volatile organic compounds (Damann et al. 2006; Inoue and Bryant 2005).

In conclusion, the TRPM5-expressing chemosensory cells found in this study provide a mechanism by which an animal can monitor quickly changes in environmental irritants. Further studies are needed to investigate signal transduction and regulation in these chemosensory cells.

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