Cross-adaptation between Olfactory Responses Induced by Two Subgroups of Odorant Molecules

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ABSTRACT It has long been believed that vertebrate olfactory signal transduction is mediated by independent multiple pathways (using cAMP and InsP₃ as second messengers). However, the dual presence of parallel pathways in the olfactory receptor cell is still controversial, mainly because of the lack of information regarding the single-cell response induced by odorants that have been shown to produce InsP₃ exclusively (but not cAMP) in the olfactory cilia. In this study, we recorded activities of transduction channels of single olfactory receptor cells to InsP₃-producing odorants. When the membrane potential was held at −54 mV, application of InsP₃-producing odorants to the ciliary region caused an inward current. The reversal potential was 0 ± 7 mV (mean ± SD, n = 10). Actually, InsP₃-producing odorants generated responses in a smaller fraction of cells (lilial, 3.4%; lyril, 1.7%) than the cAMP-producing odorant (cineole, 26%). But, fundamental properties of responses were surprisingly homologous; namely, spatial distribution of the sensitivity, waveforms, I-V relation, and reversal potential, dose dependence, time integration of stimulus period, adaptation, and recovery. By applying both types of odorants alternatively to the same cell, furthermore, we observed cells to exhibit symmetrical cross-adaptation. It seems likely that even with odorants with different modalities adaptation occurs completely depending on the amount of current flow. The data will also provide evidence showing that olfactory response generation and adaptation are regulated by a uniform mechanism for a wide variety of odorants.

KEY WORDS: InsP₃ • cAMP • olfactory receptor cell • signal transduction • adaptation

INTRODUCTION

Olfaction begins at the cilia of the receptor cell in the olfactory epithelium. Odorant binding to receptor proteins on the ciliary surface activates enzymatic cascades that lead to the opening of ionic channels, causing membrane depolarization. One well-established mechanism underlying this signal transduction involves cAMP (for reviews see Bakalyar and Reed, 1991; Breer and Boekhoff, 1992; Reed, 1992; Ronnett and Snyder, 1992; Kurahashi and Yau, 1994; Schild and Restrepo, 1998), which finally activates cyclic nucleotide–gated (CNG) cation channels and Ca²⁺-activated Cl⁻ channels (for reviews see Gold et al., 1990; Kurahashi and Yau, 1994; Firestein and Shepherd, 1995; Schild and Restrepo, 1998; Gold, 1999; Frings et al., 2000; Firestein, 2001). Another mechanism employs InsP₃ as a second messenger (Huque and Bruch 1986; Breer and Boekhoff, 1991; for review see Schild and Restrepo, 1998) that activates InsP₃-gated cation channel.

In the research history, the presence of multiple pathways was initially proposed by the experiment done by Sklar et al. (1986), who actually measured cAMP production biochemically with 65 different odorants in the olfactory ciliary membrane of the frog. They found that cAMP production rate varied among odorants, and further showed that certain types of odorants failed to produce cAMP. Later, those odorants (including lilial and lyril) that did not produce cAMP were found to produce InsP₃ instead (in rodent, Breer and Boekhoff, 1991). In addition, Kashiwayanagi et al. (1996) showed in the bullfrog that single-cell responses induced by odorants that were subgrouped into InsP₃- and cAMP-producing odorants did not cross-adapt with each other, and concluded that those olfactory transductions were mediated by independent pathways. Because of the consistencies of data obtained from various animals used in the past, the concept of multiple pathways has been universally applied to wide varieties of species within vertebrate, at least for volatile perceptions. For a long period of time, two transduction systems have been thought to be completely independent. However, a recent study by Vogl et al. (2000) showed that both pathways might have a cross-talk via cytoplasmic elements.

Thus, it has long been believed that olfactory receptor cells utilize multiple pathways for their signal trans-
ductions. On the other hand, however, recent studies that employed a combination of electro-olfactogram recordings and gene-targeting methods have argued against the presence of multiple pathways (Brunet et al., 1996; Belluscio et al., 1998; Wong et al., 2000). They showed that electro-olfactogram responses were disappeared or reduced in animals that lacked elements needed for cAMP metabolisms, and the presence of InsP₃ pathway is still controversial (Gold, 1999). Even after these findings, however, there are still several arguments for the possibility of the presence of the InsP₃ pathway (see e.g., Vogl et al., 2000; Kaur et al., 2001). A main reason why this issue remains unclear is a lack of detailed information regarding the response of single olfactory receptor cells to odorants that produce InsP₃ exclusively. So far, responses to odorants were mainly recorded in single olfactory receptor cells by using cAMP-producing odorants, because the responsiveness of cells has been shown to be much higher than using InsP₃-producing odorants (Lowe and Gold, 1993a). And, therefore, very little is known about the fundamental properties of membrane responses induced by InsP₃-producing odorants. In this study, we obtained membrane responses to InsP₃-producing odorants (lilial and lyral) by screening a large number (>2,800) of isolated single olfactory receptor cells under the whole-cell voltage clamp condition. We confirmed that electrophysiological properties of the membrane conductance induced by the InsP₃-producing odorants were very similar to those of the response induced by cAMP-producing odorants. Furthermore, it was found that there were cells sensitive to both InsP₃- and cAMP-producing odorants, and that these cells showed cross-adaptation to both types of odorants. It thus seems likely that olfactory adaptation occurs independently of the odorant modalities in the single cell. Our data will also add evidence supporting the notion that olfactory signal transduction is mediated by a single cytoplasmic cascade for a wide variety of odorants.

MATERIALS AND METHODS

Preparation

Olfactory receptor cells were dissociated enzymatically from the olfactory epithelium of the newt, Cynops pyrrohogaster. The experiments were performed under the latest ethical guidelines for animal experimentation at Osaka University, based on international experimental regulations. Dissociation protocols have been described elsewhere (Kurashashi, 1989; Hirono et al., 1992). In short, the animal was chilled on ice and double pithed. The mucosa excised from the olfactory cavity were incubated for 5 min at 37°C in a solution which contained enzymes (0.1% collagenase or 0.3% papain and 0.3% collagenase) with no added Ca²⁺ and Mg²⁺ (in mM): 110 NaCl, 3.7 KCl, 10 HEPES, 15 glucose, 1 pyruvate. In some experiments the tissue was further treated by a solution containing 0.1% DNase for 1.5 min to remove DNA filaments that interfere with the formation of giga-ohm seal. We did not see any remarkable differences in data between preparations with and without DNase. All solutions were adjusted to pH 7.4 with NaOH. The tissue was rinsed three times with normal Ringer’s solution (in mM): 110 NaCl, 3.7 KCl, 3 CaCl₂, 1 MgCl₂, 10 HEPES, 15 glucose, 1 pyruvate, and triturated. Isolated cells were plated on the concanavalin A-coated glass coverslip. Cells were maintained at 4°C until use. In this study, we selected olfactory receptor cells having more than five cilia.

Recording Procedures

Membrane currents were recorded with the whole-cell recording configuration (Hamill et al., 1981). Patch pipettes were made of borosilicate tubing with filament (outer diameter, 1.5 mm; World Precision Instruments) by using a two-stage vertical patch electrode puller (PP-830; Narishige Scientific Instruments). The recording pipette was filled with a solution containing: (in mM) 119 CaCl₂, 1 CaCl₂, 5 EGTA, 10 HEPES (pH was adjusted for 7.4 by COOH) to suppress K channels that cause large current fluctuations. Normal Ringer’s solution (for composition, see above) was used as the external solution for all recordings. The pipette resistance was 10–15 MΩ. The membrane potentials were corrected for by the liquid junction potential at the pipette tip (4 mV, see Kurashashi, 1989).

The recording pipette was connected to a patch clamp amplifier (Axopatch 1B, 1D or 200B; Axon Instruments, Inc.). The signal was low-pass filtered at 0.5 kHz, digitized by an A/D converter (sampling frequency, 1 kHz) connected to a computer (PC9821; NEC or IBM compatible PC). Simultaneously, signals were monitored on an oscilloscope and recorded on a chart recorder. Odor stimuli and the data acquisition were regulated by the same computer using a combination of original program and pClamp (ver. 6.0, Axon Instruments, Inc.). The results were analyzed by an offline computer and plotted by using Microcal Origin 6.0 software (OriginLab Corporation). For curve drawings, data sampled at 1/16 kHz were used. Experiments were performed at room temperature (23–25°C).

Odorant Stimulation

Odorants (cineole; eukalyptos commerce by Katayama Chemical Co. lilial and lyral, donated by Takasago Co.) were dissolved in DMSO and then diluted in the bath solution at 1 mM concentration with final DMSO concentration 0.2%. The stimulus solution was applied to the cilia from two independent puffer pipettes having a tip diameter of 1 μm. The tips of the puffer pipettes were, unless otherwise indicated, situated ~20 μm from the tip of the dendrite. The pressure (maximum 150 kPa) was controlled by a computer-regulated pressure ejection system developed in our laboratory (Ito et al., 1995). There was a time lag between the actual chemical stimulation of the cell and the application of TTL pulse to the magnetic valve. This time lag, being 60 ms on average, was estimated from the liquid junction current caused by the same application system.

RESULTS

The Response Induced by InsP₃-producing Odorants

Under the voltage clamp condition, puff application of InsP₃-producing odorants (either 1 mM lilial alone or 1 mM lyral alone, or a mixture of both) induced an inward current at ~54 mV (Fig. 1). The amplitude of the inward current was 100 ± 110 pA (maximum value obtained in individual cells, mean ± SD, n = 111, ranging from 2.5 to 415 pA). With a cAMP-producing odorant
(1 mM cineole), we obtained inward current responses of $215 \pm 163$ pA ($n = 329$, ranging from 9 to 724 pA). Thus, on average, responses induced by InsP$_3$-producing odorants were relatively smaller in amplitude than responses induced by the cAMP-producing odorant.

**Heterogeneous Responsiveness of Cells to Odorants**

As visualized in Fig. 2, the probability that cells generate responses to InsP$_3$-producing odorants was much smaller when compared with that obtained with a cAMP-producing odorant (cineole). This observation is actually consistent with a previous report that olfactory field potential (electro-olfactogram) responses are smaller with InsP$_3$-producing odorants than with cAMP-producing odorants (Lowe et al., 1989). By applying both lilial and cineole to the same cell, we could observe cells that responded to both InsP$_3$- and cAMP-producing odorants (Fig. 2 B). This observation was unpredictable, because it has been shown that both cAMP and InsP$_3$ pathways use independent molecules (for review see Schild and Restrepo, 1998), and that individual receptor cells express only one type of receptor protein on their plasma membrane (Ngai et al., 1993; Ressler et al., 1993; Vassar et al., 1993; Chess et al., 1994). It seems likely that certain types of receptor protein can recognize both InsP$_3$- and cAMP-producing odorants (see also Kajiya et al., 2001).

It was interesting to note that cineole responses were observed more frequently within lilial-sensitive cells than in lilial-insensitive cells (Fig. 2 C), and vice versa that lilial responses were recognized more frequently in cineole-sensitive cells than in cineole-insensitive cells (Fig. 2 D). These results suggest that there is a positive cross-correlation between lilial and cineole sensitivities among cells.

**Response Shape**

The shape of responses induced by InsP$_3$-producing odorants was very similar to that of responses induced by cAMP-producing odorants (Fig. 3). The inward current induced by InsP$_3$-producing odorants started to develop $247 \pm 115$ ms ($n = 69$) after the onset of brief
Spatial Distribution of the Sensitivity

Cells’ sensitivity to InsP$_3$-producing odorants was the maximum at the apical dendritic part (Fig. 1) where sensory cilia were elongated. When the tip of the puffer pipette (~1 μm opening diameter) was positioned close to the apical dendrite, the amplitude of the inward current was ~140 pA. As the tip was shifted to proximal part, the amplitude of the inward current reduced, and became almost undetectable when the tip was positioned at proximal part of the cell body. Furthermore, the response latency was shortest when the odorant pulse (50–100-ms duration). This value was almost comparable to that observed for the cAMP metabolism in the olfactory transduction in which the response latency attributes to the time needed for cytoplasmic cAMP concentration to increase high enough to drive CNG channels (Takeuchi and Kurahashi, 2002). The time to peak was ~0.3 s (Fig. 3 F). After the peak, the inward current in turn gradually returned to zero level within a couple of seconds (Fig. 3 H). It was noticeable to see that the response lasted much longer than the period that the cell was exposed to the stimulant. In other words, the machinery activated by InsP$_3$-producing odorants had ability for time amplification of the olfactory signal. In the cAMP pathway, time amplification of signal has been shown to be achieved by the prolonged activation of adenyl cyclase and a prolonged cAMP lifetime (Takeuchi and Kurahashi, 2002). Similarity of response shapes between InsP$_3$ and cAMP-producing odorants is also reported for olfactory cells from the tiger salamander (Chen et al., 2000).

Voltage and Dose Dependence of Odorant-induced Current

The amplitude and polarity of the current induced by InsP$_3$-producing odorants were dependent on the holding voltage ($V_h$) (Fig. 4). At negative potentials, the current polarity was inward. As the membrane potential was shifted to positive, the amplitude of the inward current reduced. Current reversal occurred at $0 \pm 7$ mV ($n = 10$). The I-V relation showed slight outward rectification (Fig. 4 B). The reversal potential and the shape of the I-V relation were all very similar to those reported previously for the current activated by cAMP-producing odorants (Kurahashi, 1989; Lowe and Gold, 1993a).

Furthermore, it was frequently observed that the current falling phase became more gradual at positive potentials. This phenomenon has been also reported for responses induced by cAMP-producing odorants (Kurahashi and Shibuya, 1990; Lowe and Gold, 1993a). In the cAMP system the voltage dependence of the current shape has been explained by the contribution of Ca$^{2+}$ feedback system on the current falling phase; the driving
The force for Ca\(^{2+}\) entry through the ion channels becomes bigger in proportion to the membrane hyperpolarization. The response amplitude was dependent on the dose of odorant stimulation (Fig. 5). With a fixed duration of 50 ms, an increase in the pressure applied to puffer pipette increased the current amplitude monotonically. One noticeable feature was that the slope of rising phase became steeper as the odorant pressure was increased (Fig. 5 A). The relation between response amplitude and pressure could be fitted by the Hill equation with high cooperativity (Hill coefficient, \(n = 5.4\), \(n = 4.3\)), as has been reported for the olfactory receptor cell responding to cAMP-producing odorants (Lowe and Gold, 1993b).

The amplitude of current responses was dependent also on the duration of the odorant stimuli at fixed pressure (Fig. 5 C). The relation between current amplitude and stimulus duration could be fitted by the Hill equation with high cooperativity (Hill coefficient, \(n_H = 5.4 \pm 4.3\), \(n = 10\)), as has been reported for the olfactory receptor cell responding to cAMP-producing odorants (Lowe and Gold, 1993b).

The amplitude of current responses was dependent also on the duration of the odorant stimuli at fixed pressure (Fig. 5 C). The relation between current amplitude and stimulus duration was also fitted by the Hill equation (Fig. 5 B), with a Hill coefficient of \(2.4 \pm 0.8\) (\(n = 5\)). It is thus likely that the response generation system has an ability to integrate the stimulus period, as has been reported for the cAMP cascades (Firestein et al., 1993; Takeuchi and Kurahashi, 2002).

**Self Adaptation to InsP\(_3\)-producing Odorants**

When cells were stimulated by double pulse protocols, the response induced by the second stimulus showed remarkable reduction in amplitude in comparison with the first response induced by a conditioning pulse (Fig. 6). This phenomenon is very similar to that reported for olfactory adaptation analyzed with cAMP-producing odorants (Kurahashi and Menini, 1997).

With InsP\(_3\)-producing odorants, reduction rate was 37 ± 14% (\(n = 8\)) when the second pulse was applied immediately after the first response was terminated. As the interstimulus interval was prolonged, the current amplitude gradually recovered. Full recovery was established \(~10–20\) s after the onset of the conditioning pulse. These parameters relating adaptation were almost the same as those observed in responses induced by cAMP-producing odorants (see Kurahashi and Shibuya, 1990; Kurahashi and Menini, 1997).

**Cross-adaptation between InsP\(_3\) and cAMP-producing Odorants**

As mentioned before, we could find cells that responded to both InsP\(_3\)- and cAMP-producing odorants. When both types of odorants were alternatively applied to the same cell as a double pulse protocol, cells expressed “cross-adaptation” (Fig. 7); the response induced by the InsP\(_3\)-producing odorant was reduced by the conditioning pulse using cAMP-producing odorant, and the response induced by the cAMP-producing odorant was reduced by the InsP\(_3\)-producing odorant.
degree of reduction more quantitatively, we first adjusted the amplitudes of responses induced by both InsP₃- and cAMP-producing odorants to the same levels, and then those odorants were applied alternatively to the same cell. Interestingly, the second responses reduced to the same level, independent of the odorant species applied as conditioning pulse. Furthermore, the recovery phase was again the same (Fig. 8). The most likely explanation for these results would be that both types of odorants activate the same transduction machinery. Further discussion on this matter will be made later.

**DISCUSSION**

In this study, we obtained responses induced by InsP₃-producing odorants (lilial and lyral) in single olfactory receptor cells. Fundamental properties, which include spatial distribution of the sensitivity, waveforms, I-V relation and reversal potential, dose dependence, time integration of stimulus period, adaptation, and recovery, were almost identical to those of responses induced by cAMP-producing odorants. A remarkable difference observed between InsP₃- and cAMP-producing odorants was the probability of response generation; cineole
responses were observed in 26% of cells, while lilial and lyral induced responses in 3.4% and 1.7% of cells, respectively. By applying both types of odorants alternatively to the same cell, furthermore, we observed cells to exhibit symmetrical cross-adaptation. Thus, it seems likely that, even with odorants with different modalities, adaptation occurs completely depending on the amount of current flow. This could be actually predicted by previous observation that CNG channel is the target of Ca\(^{2+}\)/H\(^{+}\)-feedback expressing adaptation (Kurahashi and Menini, 1997).

**Heterogeneous Sensitivities to Odorants in Relation to the Expression of Receptor Proteins**

In the present study, we saw that the responsiveness of cells to odorants were heterogeneous. Looking at selectivity between lilial (InsP\(_3\)-producing odorant) and cineole (cAMP-producing odorant), all four possible combinations were observed; namely, cells responding to lilial alone, to cineole alone, cells responding to both, and to neither. However, it is notable to see that the cineole response was observed more frequently in cells responding to lilial (38%) than in cells not responding to lilial (26%). And, vice versa, lilial response was observed more frequently in cells responding to cineole. This may suggest that, although cineole and lilial are subgrouped into cAMP- and InsP\(_3\)-producing odorants, respectively, there are some positive correlation in cells’ sensitivities. Since a cell expresses only one type of the receptor protein as predicted from molecular biological investigations (Ngai et al., 1993; Ressler et al., 1993; Vassar et al., 1993; Chess et al., 1994), these observations suggest that the receptor protein that accepts lilial is much more sensitive to cineole than the receptor protein insensitive to lilial.

The fraction of cells responding to InsP\(_3\)-producing odorants was very small in comparison with the occurrence of cineole responses. This is consistent with a previous observation that the electrical field potential (electro-olfactogram) is smaller in amplitude for InsP\(_3\)-producing odorants (Lowe et al., 1989). Amplitude of electro-olfactogram reflects a combination of response amplitude in individual cells and the number of cells responding. The result obtained in this study clearly shows that the number of cells responding to InsP\(_3\)-producing odorants is smaller than the number of cells responding to cAMP-producing odorants. Obviously, cells expressing odorant receptors that accept InsP\(_3\)-producing odorants are the minority.

**Reversal Potential and Ionic Selectivity**

The reversal potential is an index to estimate ionic selectivity of ionic channels generating currents. The InsP\(_3\)-producing odorant response showed the reversal potential near 0 mV. This indicates that the underlying ionic channel is either cation and/or anion permeable. Although our present experiments do not address the ionic species directly, the reversal potential value is consistent with previous observations for the InsP\(_3\)-activated channels to be a cation selective (Schild et al., 1995; Schild and Restrepo, 1998; Kaur et al., 2001). Furthermore, CNG channels are also cation selective (Kurahashi and Shibuya, 1990).

It is also reported that Ca\(^{2+}\) entry through the InsP\(_3\)-activated cation channels can activate K-selective channels (Morales et al., 1997). In the present study, however, we used Cs pipette solution, and therefore cannot
Cross-adaptation between Different Odorants

The response induced by InsP₃-producing odorants showed a very similar waveform to the response induced by cAMP-producing odorants. This result actually argues against the notion that responses induced by InsP₃- and cAMP-producing odorants are mediated by multiple pathways. It is generally figured out that cAMP and InsP₃ pathways utilizes completely independent molecular components; namely, the cAMP pathway uses receptors, G_o₉, type III adenylyl cyclase, cAMP, and CNG channels; the InsP₃ pathway uses receptors, G_o₉, phospholipase C, InsP₃, and InsP₃-gated cation channels. Any dynamic parameters of these elemental molecules could not be identical. Reactions and activated time courses of the enzymes would be different. Temporal patterns of cAMP and InsP₃ formations, movements, and their hydrolyses cannot be identical. In the present study, however, the final output (channel activity) showed the same waveform in terms of the response latency, time to peak, and response shutdown. In general, these results would suggest that responses induced by two groups of odorants are mediated by the same second messenger system. Further quantitative information for the molecular elements underlying both systems would be indispensable to completely understand if responses are mediated by two independent pathways.

**Ca Dynamics**

In the olfactory receptor cell it has been well known that cytoplasmic Ca²⁺ plays key roles regulating adaptation (Kurahashi and Menini, 1997) and signal amplification via an activation of Ca²⁺-activated Cl⁻ channel (Lowe and Gold, 1993b). Because of its importance, Ca²⁺ homeostasis is thought to be balanced in olfactory cilia by very critical regulatory systems; namely, a complex of molecular networks involving the Ca²⁺ influx through the transduction channels, the Na⁺-dependent Ca²⁺ extrusion system (Reisert and Matthews, 2001), and, presumably, the Ca²⁺-buffering system. Therefore, it is expected that the waveform of the odorant-induced current is not only regulated by the enzymes involving transduction, but also by the Ca²⁺ metabolisms. In the present study, we observed that responses induced by both categories of odorants expressed similar waveforms of responses. To express the same waveforms of responses with different transduction channels (InsP₃-gated channels and CNG channels), both channels must be identical critically in terms of the Ca²⁺ permeability, activation, and shut-off kinetics. This would be very hard to imagine with a simple coincidence.

**Self-adaptation and Cross-adaptation with cAMP-producing Odorants**

Responses induced by InsP₃-producing odorants expressed adaptation when examined by double pulse protocols. Adaptation of responses induced by cAMP-producing odorants has been shown to be regulated by the Ca²⁺-feedback to CNG channels (Kurahashi and Menini, 1997). Recovery from adaptation is regulated by the Na⁺-dependent Ca²⁺ extrusion system (Reisert and Matthews, 2001), and, presumably, the Ca²⁺-buffering system. Therefore, it is expected that the waveform of the odorant-induced current is not only regulated by the enzymes involving transduction, but also by the Ca²⁺ metabolisms. In the present study, we observed that responses induced by both categories of odorants expressed similar waveforms of responses. To express the same waveforms of responses with different transduction channels (InsP₃-gated channels and CNG channels), both channels must be identical critically in terms of the Ca²⁺ permeability, activation, and shut-off kinetics. This would be very hard to imagine with a simple coincidence.

**Figure 7. Cross-adaptation between InsP₃- and cAMP-producing odorants.**

(A) Lilial stimulation was applied after the stimulation by cineole. Interval of stimuli were 2 s. Prior to the present experiment, maximum peaks of responses induced by lilial and cineole were adjusted to the same by controlling pressures applied to puffer pipettes. Cineole stimulus; pressure, 100 kPa, duration 100 ms. Lilial; pressure, 20 kPa, duration 100 ms. V_h = -54 mV. (B) Lilial stimulation was applied after the cineole pulse. (C) Superimpose of data from A and B. Note that the reduction in the amplitude is identical in these conditions.

The answer to the question if K_(Ca) component was included in responses induced by InsP₃-producing odorants.
dependent mechanism. It is unlikely that, in addition
to discussion made in previous sections, different sys-
tems can express the same adaptational time courses
(reduction rate, time course for recovery).

Recently, Vogl et al. (2000) showed that InsP3 and
cAMP pathways could suppress each other. Qualita-
tively, this finding seems likely to explain our observa-
tion of cross-adaptation. But, in our experiments, the
reduction rate matched perfectly whether we used
InsP3- or cAMP-producing odorant as a conditioning
pulse. Coupled with step-by-step considerations de-
scribed above, present observations would satisfac-
tory lead us to conclude that responses induced by both
InsP3- and cAMP-producing odorants in the same cell
were mediated by the same system. Furthermore, it is
highly likely that the mechanism underlying adapta-
tion is uniform. However, our results do not exclude
the possibility that InsP3-producing odorants produce
InsP3. And, therefore, the findings by Vogl et al. (2000)
may raise a new possibility that InsP3 is actually used for
modulating the olfactory signal transduction.

This study showed that fundamental properties of
single-cell responses induced by InsP3-producing odor-
ants were very similar to those observed for responses
induced by cAMP-producing odorants. As mentioned
at the beginning of RESULTS, on average, amplitude of
responses induced by InsP3-producing odorants was
about half of that of cineole response. This statistical
difference may be explained by assuming that odorants
that have been thought to stimulate the InsP3 pathway
indeed activate the cAMP metabolism with lower effi-
ciency than the other odorants. The data obtained
here would provide evidence, in addition to experi-
ments done by Gold and his collaborators (Brunet et
al., 1996; Belluscio et al., 1998; Wong et al., 2000) and
Zufall's group (Chen, et al., 2000), showing that the re-
response induced by InsP3-producing odorants is medi-
ated by the cAMP pathway.

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REFERENCES

Bakalyar, H.A., and R.R. Reed. 1991. The second messenger cas-
cade in olfactory receptor neurons. Curr. Opin. Neurobiol. 1:204–
208.

Belluscio, L., G.H. Gold, A. Nemes, and R. Axel. 1998. Mice defi-
cient in G(olf) are anosmic. Neuron. 20:69–81.

Breer, H., and I. Boekhoff. 1991. Odorants of the same odor class
activate different second messenger pathways. Chem. Senses. 16:
19–29.

Breer, H., and I. Boekhoff. 1992. Second messenger signaling in ol-
faction. Curr. Opin. Neurobiol. 2:439–443.

Brunet, L.J., G.H. Gold, and J. Nigai. 1996. General anosmia caused
by a targeted disruption of the mouse olfactory cyclic nucleotide-
gated cation channel. Neuron. 17:681–693.

Chen, S., A.P. Lane, R. Bock, T. Leinders-Zufall, and F. Zufall. 2000.
Blocking adenyl cyclase inhibits olfactory generator current
duced by IP(3)-odors. J. Neurophysiol. 84:575–580.

Chess, A., I. Simon, H. Cedar, and R. Axel. 1994. Allelic inactivation
regulates olfactory receptor gene expression. Cell. 78:823–834.

Firestein, S. 2001. How the olfactory system makes sense of scents.
Nature. 413:211–218.

Firestein, S., C. Picco, and A. Menini. 1993. The relation between
stimulus and response of olfactory receptor cells of the tiger salamander. J. Physiol. 468:1–10.

Firestein, S., and G.M. Shepherd. 1995. Interaction of anionic and cationic currents leads to a voltage dependence in the odor response of olfactory receptor neurons. J. Neurophysiol. 73:562–567.

Frings, S., D. Reuter, and S.J. Kleene. 2000. Neuronal Ca\(^{2+}\)-activated Cl\(^{-}\) channels homing in on an elusive channel species. Prog. Neurobiol. 60:247–289.

Gold, G.H. 1999. Controversial issues in vertebrate olfactory transduction. Annu. Rev. Physiol. 61:857–871.

Gold, G.H., T. Nakamura, and G. Lowe. 1990. Studies on the mechanism of olfactory transduction in vertebrates. Neur. Res. Suppl. 12:S127–S134.

Hamill, O.P., A. Marty, E. Neher, B. Sakmann, and F.J. Sigworth. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches.

Kajiyama, M. Tonoike, and M. Takebayashi. 1992. Simultaneous recording of [Ca\(^{2+}\)]\(i\) increases in isolated olfactory receptor neurons retaining their original spatial relationship in intact tissue. J. Neurosci. Methods. 42:185–194.

Huque, T., and R.C. Bruch. 1986. Odorant- and guanine nucleotide-stimulated phospholipid turnover in olfactory cilia. Biochem. Biophys. Res. Commun. 137:36–42.

Ito, Y., T. Kurahashi, and A. Kaneko. 1995. Pressure control instrumentation for drug stimulation. Nippon Seirigaku Zasshi. 57:127–133.

Kaur, R., X.O. Zhu, A.J. Moorhouse, and P.H. Barry. 2001. IP3-gated channels and their occurrence relative to CNG channels in olfactory receptor cells. Membr. Biol. 181:91–105.

Kashiwayanagi, M., K. Shimano, and K. Kurihara. 1996. Existence of multiple receptors in single neurons: responses of single bullfrog olfactory neurons to many cAMP-dependent and independent odorants. Brain Res. 738:222–228.

Kaur, R., T.S. Wong, B. Hacker, G.C. Chan, G. Lowe, A. Gaggar, Z. Xia, G.H. Gold, and D.R. Storm. 2000. Disruption of the type III adenylyl cyclase gene leads to peripheral and behavioral anosmia in transgenic mice. Neuron. 27:487–497.

Kurahashi, T. 1989. Activation by odorants of cation-selective conductance in the olfactory receptor cell isolated from the newt. J. Physiol. 419:177–192.

Kurahashi, T., and A. Kaneko. 1991. High density cAMP-gated channels at the ciliary membrane in the olfactory receptor cell. Neuron. 5:5–8.

Kurahashi, T., and A. Kaneko. 1993. Gating properties of the cAMP-gated channel in toad olfactory receptor cells. J. Physiol. 466:287–302.

Kurahashi, T., and A. Menini. 1997. Mechanism of odorant adaptation in the olfactory receptor cell. Nature. 385:725–729.

Kurahashi, T., and T. Shibuya. 1990. Ca\(^{2+}\)-dependent adaptive properties in the solitary olfactory receptor cell of the newt. J. Physiol. 515:261–268.

Kurahashi, T., and K.W. Yau. 1994. Olfactory transduction. Tales of an unusual chloride current. Curr. Biol. 4:256–258.

Larsson, H.P., S.J. Kleene, and H. Lecar. 1997. Noise analysis of ion channels in non-space-clamped cables: estimates of channel parameters in olfactory cilia. Biophys. J. 72:1193–1203.

Lowe, G., and G.H. Gold. 1991. The spatial distributions of odorant sensitivity and odorant-induced currents in salamander olfactory receptor cells. J. Physiol. 442:147–168.

Lowe, G., and G.H. Gold. 1993a. Contribution of the ciliary cyclic nucleotide-gated conductance to olfactory transduction in the salamander. J. Physiol. 462:173–196.

Lowe, G., and G.H. Gold. 1993b. Nonlinear amplification by calcium-dependent chloride channels in olfactory receptor cells.

Lowe, G., T. Nakamura, and G.H. Gold. 1989. Adenylyl cyclase mediates olfactory transduction for a wide variety of odorants. Proc. Natl. Acad. Sci. USA. 86:5641–5645.

Morales, B., R. Madrid, and J. Bacigalupo. 1997. Calcium mediates the activation of the inhibitory current induced by odorants in toad olfactory receptor neurons. FEBS Lett. 402:259–264.

Ngai, J., M.M. Dowling, L. Buck, R. Axel, and A. Chess. 1995. The family of genes encoding odorant receptors in the channel catfish. Cell. 72:657–666.

Reed, R.R. 1992. Signaling pathways in odorant detection. Neuron. 8:209–209.

Reisert, J., and H.R. Matthews. 2001. Response properties of isolated mouse olfactory receptor cells. J. Physiol. 530:113–122.

Ressler, K.J., S.L. Sullivan, and L.B. Buck. 1993. A zonal organization of odorant receptor gene expression in the olfactory epithelium. Cell. 73:597–609.

Ronnett, G.V., and S.H. Snyder. 1992. Molecular messengers of olfaction. Trends Neurosci. 15:508–513.

Schild, D., F.W. Lischka, and D. Restrepo. 1995. InsP3 causes an increase in apical [Ca\(^{2+}\)]\(i\) by activating two distinct components in vertebrate olfactory receptor cells. J. Neurophysiol. 73:862–866.

Schild, D., and D. Restrepo. 1998. Transduction mechanisms in vertebrate olfactory receptor cells. Physiol. Rev. 78:429–466 (Review).

Sklar, P.B., R.R. Anhalt, and S.H. Snyder. 1986. The odorant-sensitive adenylyl cyclase of olfactory receptor cells. Differential stimulation by distinct classes of odorants. J. Biol. Chem. 261:15538–15543.

Takeuchi, H., and T. Kurahashi. 2002. Photolysis of caged cyclic AMP in the ciliary cytoplasm of the newt olfactory receptor cell. J. Physiol. 541:825–833.

Vassar, R., J. Ngai, and R. Axel. 1993. Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. Cell. 74:309–318.

Vogl, A., J. Noe, H. Breer, and I. Boekhoff. 2000. Cross-talk between olfactory second messenger pathways. Eur. J. Biochem. 267:4529–4535.

Wong, S.T., K. Trinh, B. Hacker, G.C. Chan, G. Lowe, A. Gaggar, Z. Xia, G.H. Gold, and D.R. Storm. 2000. Disruption of the type III adenylyl cyclase gene leads to peripheral and behavioral anosmia in transgenic mice. Neuron. 27:487–497.