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Inward currents and increases in cytosolic Ca^{2+} concentration induced by cyclic ADP-ribose in turtle olfactory receptor cells

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Abstract

In olfactory receptor cells, it is well established that cyclic AMP (cAMP) and inositol-1,4,5-trisphosphate (IP_3) act as second messengers during odor responses. In previous studies, we have shown that cAMP-increasing odorants induce odor responses even after complete desensitization of the cAMP-mediated pathway. These results suggest that at least one cAMP-independent pathway contributes to the generation of odor responses. In an attempt to identify a novel second messenger, we investigated the possible role of cyclic ADP-ribose (cADPR) in olfactory transduction. Turtle olfactory receptor cells were isolated using an enzyme-free procedure and loaded with fura-2/AM. The cells responded to dialysis with cADPR with an inward current and an increase of the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$. Flooding of cells with 100 μM cADPR from the pipette also induced an inward current without changes in $[Ca^{2+}]_i$ in Na^+-containing and Ca^{2+}-free Ringer solution. In an Na^+-free and Ca^{2+}-containing Ringer solution, cADPR induced only a small inward current with a concomitant increase in $[Ca^{2+}]_i$. Inward currents and increases in $[Ca^{2+}]_i$ induced by cADPR were completely inhibited by removal of both Na^+ and Ca^{2+} from the outer solution. The experiments suggest that cADPR activates a cation channel at the plasma membrane, allowing inflow of Na^+ and Ca^{2+} ions. The magnitudes of the inward current responses to cAMP-increasing odorants were greatly reduced by prior dialyses of a high concentration of cADPR or 8-bromo-cyclic ADP-ribose (8-Br-cADPR), an antagonist. It is possible that the cADPR-dependent pathway contributes to the generation of olfactory responses.

Key words: Ca^{2+}, cyclic ADP-ribose, inward current, odor response, olfactory cell

Introduction

It has been well established that cyclic AMP (cAMP) acts as a second messenger in olfactory transduction. There is good evidence that the binding of one set of chemical stimuli to odorant receptors leads to stimulation of adenylyl cyclase via GTP-binding protein (G protein) (Pace *et al.*, 1985; Sklar *et al.*, 1986; Breer and Boekhoff, 1991). In turn, an increase in the cAMP level activates cAMP-gated cation channels (Nakamura and Gold, 1987; Suzuki, 1987; Kurahashi, 1990), causing cell depolarization and a discharge of action potentials (Trotier, 1986; Frings *et al.*, 1991). We have previously shown that cAMP-increasing odorants induce a large olfactory response, even after complete desensitization of the cAMP-dependent pathway (Kashiwayanagi *et al.*, 1994; Kashiwayanagi and Kurihara, 1995). These results suggested that cAMP-independent pathways contribute to the generation of odor responses. Until now, however, it has remained unclear as to how odor responses are generated via the cAMP-independent pathway.

In several cases, odorant-induced responses are accompanied by increases in cytosolic Ca^{2+} concentrations ($[Ca^{2+}]_i$) and intraciliary Ca^{2+} concentrations (Sato *et al.*, 1991; Restrepo *et al.*, 1993b; Kashiwayanagi and Lindemann, 1995; Leinders-Zufall *et al.*, 1998). Cyclic ADP-ribose (cADPR), which is converted from nicotinamide adenine dinucleotide (NAD^+) by ADP-ribosyl cyclase, has been shown to mobilize Ca^{2+} in various cells, including central and peripheral neurons (Berridge, 1993; Guse, 1999; Galione *et al.*, 1991). It is possible that odorant-induced cADPR may contribute to $[Ca^{2+}]_i$ increases in olfactory receptor cells. In the present study, we found that the flooding of turtle olfactory cells with cADPR from a patch pipette induces inward currents, and that inward currents in response to cAMP-increasing odorants can be greatly reduced by prior dialysis with high concentrations of cADPR or 8-Br-cADPR, an antagonist (Guse *et al.*, 1995). It is possible that this cADPR-dependent pathway contributes to the generation of odor responses.

Methods

All experiments were carried out in accordance with the Guidelines for the Use of Laboratory Animals of the

Graduate School of Pharmaceutical Sciences, Hokkaido University.

Animals

Turtles, *Geoclemys reevesii*, weighing 150–300 g, were obtained from commercial suppliers and maintained at 25°C. Animals were fed porcine and bovine liver *ad libitum*. For the isolation of olfactory receptor cells, animals were cooled to 0°C and decapitated. The nasal cavities were opened, and the olfactory epithelia were quickly removed.

Isolation of olfactory receptor cells

The olfactory receptor cells were isolated as described previously (Kashiwayanagi *et al.*, 1994). The epithelia were cut into slices of ~150–200 μm thickness in normal Ringer solution at 0°C and stored at 4°C. Slices were incubated for 2 h at 37°C in Ca^{2+}-free Ringer solution for the isolation of olfactory receptor cells. Immediately prior to recording, one slice of the epithelium was placed in 500 μl normal Ringer solution in a recording chamber and shaken. No enzymes (such as proteases) were added. Once cells had settled on the bottom of the chamber and a tight seal had been established on a cell, the chamber was continuously perfused with normal Ringer solution. Turtle olfactory receptor cells were readily distinguished from other types of cells (e.g. respiratory and basal cells) based on their characteristic morphology, such as possessing several long motile cilia.

Whole-cell recordings

The conventional whole-cell patch clamp method was used to measure transmembrane currents (Hamill *et al.*, 1981). Recordings were made with an Axopatch 1D amplifier (Axon Instruments, Inc., Burlingame, CA) using patch electrodes of borosilicate glass with an inner filament (GD 1.5, Narishige Co., Tokyo, Japan), which were sealed on the cell body. The holding potential was –70 mV. Electrodes with a resistance of 5–10 MΩ were manufactured on a Narishige PP83 puller (Narishige Co.) using a double stage pull. Gigaohm seals were obtained by applying negative pressure (–30 to –100 cmH_2O). The whole-cell configuration was attained by the application of additional negative pressure. The current signal was digitized and stored on videotape.

Stimulation

Isolated olfactory receptor cells were irrigated by extracellular solutions according to the method described by Frings and Lindemann (Frings and Lindemann, 1991). Gravity was used to deliver a constant stream of extracellular solutions from the irrigating tube. Extracellular solutions were switched by four electrically actuated valves. The stimulating tube, which had a lumen 160–200 μm in diameter, was placed under visual control within ~500 μm of the cell. The delay due to dead space was 1–40 s, depending on the flow rate and the distance between cells and the tip of the tube.

Recording of $[Ca^{2+}]_i$

Fura-2/AM of 10 μM was loaded for 30–60 min at room temperature. An inverted microscope (Aviovert 135, Carl Zeiss, Jena, Germany) was used with a Fluor ×100 objective (Carl Zeiss). Pulses of excitation light (340 and 380 nm) were coupled to the microscope with a light guide. Images were recorded with Attofluor™ ICCD camera (Carl Zeiss). Concentrations of free cytosolic Ca^{2+} were recorded under the whole-cell voltage-clamp condition (holding potential: –70 mV), and were calculated from pixel ratios by Attofluor Ratio Vision software. *In vitro* calibration curves were obtained with calibration buffer solutions.

Preparation of solutions

Normal Ringer solution contained (mM) 116 NaCl, 4 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 15 glucose, 5 sodium pyruvate and 10 *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)–NaOH (pH 7.4). Na^+-free Ringer solution contained (mM) 116 *N*-methyl-D-glucamine (NMDG), 4 KCl, 2 $CaCl_2$, 2 $MgCl_2$, 15 glucose and 10 HEPES–NaOH (pH 7.4). Ca^{2+}-free Ringer solution contained (mM) 116 NaCl, 4 KCl, 2 $MgCl_2$, 15 glucose and 10 HEPES–NaOH (pH 7.4). Na^+-, Ca^{2+}-free Ringer solution contained (mM) 116 *N*-methyl-D-glucamine (NMDG), 4 KCl, 2 $MgCl_2$, 1 EGTA, 15 glucose and 10 HEPES–NaOH (pH 7.4). Patch pipettes were filled with an inner solution (mM): 120 K acetate, 2 $MgCl_2$, 5 ATP and 10 HEPES–KOH (pH 7.4). For the stimulation with cADPR and 8-Br-cADPR from the patch pipette, chemicals of varying concentrations were dissolved in the inner solution. The odorant cocktails were vigorously stirred with the magnetic stirrer for >30 min at room temperature. The final concentration of each odorant in cAMP-increasing odorant cocktails I (citralva, hedione, eugenol, L-carvone and cineole) and II (L-citronellal, geraniol and menthone), which increased the cAMP concentration in rat, bullfrog and turtle olfactory cells but did not change the IP_3 concentration in those of rats (Sklar *et al.*, 1986; Breer and Boekhoff, 1991; Okamoto *et al.*, 1996), was 200 μM. The final concentrations of odorants in the IP_3-increasing odorant cocktail, which increased IP_3 concentration in rat olfactory cells but not the cAMP concentration in those of rats, bullfrogs and turtles (Sklar *et al.*, 1986; Breer and Boekhoff, 1991; Okamoto *et al.*, 1996), were 20 μM lilial, 20 μM lyral and 10 μM ethyl vanillin.

Chemicals

Fura2/AM was purchased from Wako (Tokyo, Japan). All volatile odorants were kindly supplied by Takasago International (Tokyo, Japan). All chemicals used were of the highest grade available.

Results

To examine the effects of cADPR on turtle olfactory receptor cells, 100 μM cADPR was dialyzed into cells from a patch pipette. On the breaking of the patch, 100 μM cADPR induced inward currents in 22 out of 27 cells under the voltage-clamp conditions (holding potential: –70 mV). The current was desensitized in 14 cells (Figure 1A). Desensitization of the response to cADPR was slower than that to cAMP ($P < 0.001$). It took 4.9 ± 0.8 s ($n = 12$) to reduce the magnitude of the response to cADPR to 80% of the peak, whereas it took 1.8 ± 3.1 s ($n = 14$) to similarly reduce that to 1 mM cAMP (data not shown). The peak magnitude of inward currents in response to 100 μM cADPR ranged, typically, from 0 to 661 pA. The mean magnitude of the inward currents was 86.4 ± 21.5 pA ($n = 21$), which is comparable to that of the currents induced by odorants (Kashiwayanagi and Kurihara, 1995). Dialysis of the control inner solution (data not shown, $n = 17$) or 8-Br-cADPR ($n = 38$), which has a similar molecular structure to cADPR (Guse *et al.*, 1995), did not induce changes in the membrane current (Figure 2A). The voltage-dependence of 100 μM cADPR was examined by applying a voltage ramp from –120 to 80 mV (500 mV/s) to voltage-clamped cells during and after responses (Figure 1B), and our results suggest that cADPR increases membrane conductance. The slope during responses was steeper than that after responses. The mean reversal potential was estimated to be 6.0 ± 3.6 mV ($n = 10$; Figure 1C). Inward currents induced by the dialysis of cADPR reached a peak at 0.5–5 s and returned to a steady current level within ~50 s after the breakthrough. A voltage ramp was applied after 8 s (during responses) and 50 s (after responses) after the breakthrough using the patch pipette containing 8-Br-cADPR (Figure 2A). There was no significant difference in the slopes of the *I–V* curves after 8 and 50 s ($n = 9$; Figure 2B).

Figure 3A shows the membrane currents recorded from individual olfactory cells dialyzed with different concentrations of cADPR. Figure 3B shows a plot of the mean magnitudes of inward currents induced by dialysis of cADPR from the patch pipette as a function of cADPR concentrations. The magnitude of the response to cADPR increased with increases in cADPR concentration up to 100 μM but decreased above this level. It is possible that dialysis of a high concentration of cADPR induces unexpected toxic effects on olfactory cells. Therefore, we applied 100 μM cADPR in the following experiments.

cADPR has been reported to be a Ca^{2+}-mobilizer

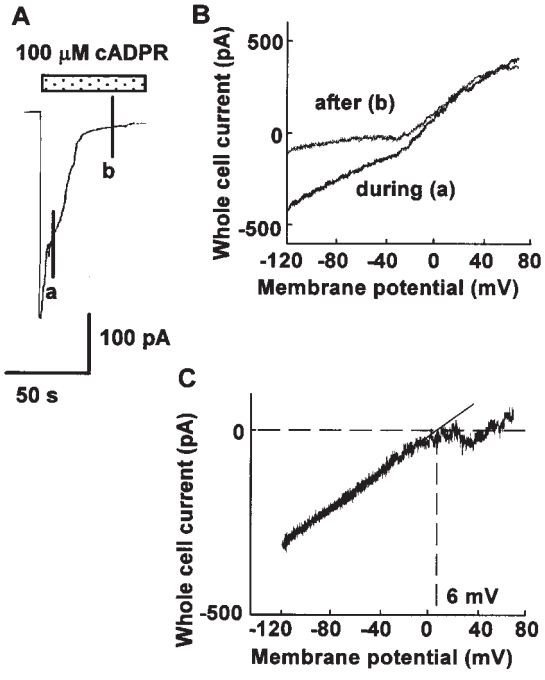

Figure 1 cADPR-induced inward currents recorded from olfactory receptor cells under the whole-cell voltage-clamp condition. **(A)** Membrane currents at the breakthrough measured with the pipette containing 100 μM cADPR under voltage-clamp with a holding potential at –70 mV. Lines a and b indicate the application of a voltage ramp. **(B)** Whole-cell current–voltage relationships for the current evoked by inner 100 μM cADPR. The current was measured by applying a voltage ramp (500 mV/s) from –120 to 80 mV during (a) and after (b) the response induced by 100 μM cADPR. **(C)** The subtraction of the *I–V* curve during the response from that after the response.

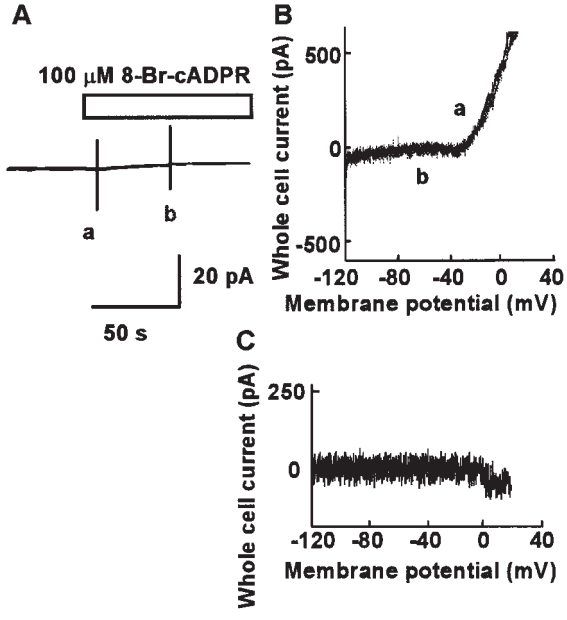

Figure 2 Effects of 8-Br-cADPR on electrical properties of membranes of olfactory cells. **(A)** Membrane currents at the breakthrough measured with the pipette containing 100 μM 8-Br-cADPR under voltage-clamp with a holding potential at –70 mV. Lines a and b indicate the application of a voltage ramp. **(B)** Whole-cell current-voltage relationships for the current evoked by inner 100 μM 8-Br-cADPR. The current was measured by applying a voltage ramp (500 mV/s) from –120 to 80 mV at 8 s (a) and 50 s (b) after the breakthrough. **(C)** The subtraction of the *I–V* curve at (a) from (b).

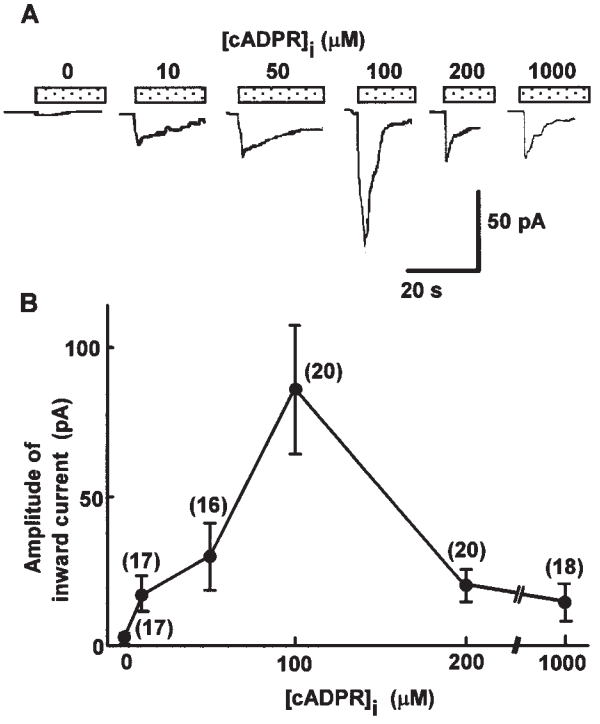

Figure 3 The dose–response relationship of inward currents induced by dialysis of cADPR. **(A)** Inward currents induced by cADPR of varying concentrations dialyzed from patch pipettes. **(B)** Magnitude of the inward currents in response to cADPR dialyzed as a function of cADPR concentration. Each point is the mean ± SE of data obtained from n preparations indicated in the figure. Holding potential was –70 mV.

(Galione *et al.*, 1991; Berridge, 1993; Guse, 1999). Hence, it is possible that cADPR also mobilizes Ca^{2+} in turtle olfactory cells. This possibility was tested by measuring cytosolic Ca^{2+} concentrations in the somas of these cells ($[Ca^{2+}]_i$) when cADPR was dialyzed under the voltage-clamp conditions in normal Ringer solution. In response to the cADPR diffusing into the cell, $[Ca^{2+}]_i$ increased with inward currents in 19 out of 22 cells. In the cell shown in Figure 4A, the increase was from 100 to ~190 nM. While the current adapted spontaneously, $[Ca^{2+}]_i$ remained high in this cell. In 3 of the 19 cells, detectable inward currents did not appear, although $[Ca^{2+}]$ did increase (data not shown), suggesting that cADPR induces a Ca^{2+} release from inner Ca^{2+} stores in these cells. The dialysis of 100 μM 8-Br-cADPR changed neither the $[Ca^{2+}]_i$ nor the membrane currents (Figure 4B).

To test the ion selectivity, changes in $[Ca^{2+}]_i$ and membrane currents in response to cADPR were measured in various outer solutions. The mean resting $[Ca^{2+}]_i$ in normal, Ca^{2+}-free, Na^+-free and Ca^{2+}-, Na^+-free Ringer solutions were 102.0 ± 9.0 (n = 18), 56.0 ± 7.0 (n = 9), 96.5 ± 6.2 (n = 24) and 53.1 ± 6.5 nM (n = 12), respectively. In Ca^{2+}-free Ringer solution, the dialysis of 100 μM cADPR from the pipette induced an inward current without changes in $[Ca^{2+}]_i$ in all eight cells (Figure 4C). The magnitude of the inward current in Ca^{2+}-free Ringer solution was ~4 times that in normal Ringer solution, suggesting that outer Ca^{2+} suppresses the inward current in response to cADPR. In Na^+-free Ringer solution, cADPR induced only small inward currents, while the magnitude of increases in $[Ca^{2+}]_i$ was similar to that in normal Ringer solution (Figure 4D). Inward currents induced by cADPR were inhibited by the substitution of Na^+ with $NMDG^+$ and the removal of free Ca^{2+} by chelating with EGTA (Figure 4E). These results suggest that cADPR activates cation channels at the plasma membranes, allowing an inflow of Na^+ and Ca^{2+}.

In Ca^{2+}-free Ringer solution and Ca^{2+}-, Na^+-free Ringer solution, cADPR did not induce a significant increase in $[Ca^{2+}]_i$, suggesting that an influx of Ca^{2+} from the extracellular solution, not a release from Ca^{2+} stores, is responsible for the increase in cytosolic Ca^{2+} concentrations under these experimental conditions.

Figure 5 summarizes changes in the membrane current and $[Ca^{2+}]_i$ induced by 100 μM cADPR in bathing solutions of different Na^+ and Ca^{2+} concentrations. cADPR did not induce significant inward currents when Na^+ was substituted with $NMDG^+$, and most of the free Ca^{2+} was chelated with EGTA. $[Ca^{2+}]_i$ increases in Ca^{2+}-free and Na^+-,Ca^{2+}-free Ringer solutions were no larger than when measured with a pipette having no cADPR, indicating that there was no large cADPR-induced Ca^{2+} release in the soma under the employed experimental conditions.

In a previous study, we showed that the application of cAMP-increasing odorants (cocktail I) after desensitization of the cAMP-dependent pathway induces a large inward current, suggesting that the cAMP-independent pathway contributes greatly to the generation of responses to cAMP-increasing odorants (Kashiwayanagi *et al.*, 1994; Kashiwayanagi and Kurihara, 1995). As shown in Figure 1, inward current responses to cADPR were desensitized when cADPR was continuously dialyzed from a patch pipette. After the 100 μM cADPR-induced inward current was adapted, the odorant cocktails were applied. The magnitudes of the responses to cAMP-increasing odorant cocktails I and II after adaptation of the cADPR-current were approximately half that recorded in the normal inner solution (Figure 6). In contrast, the response to the IP_3-increasing odorant cocktail was not changed by cADPR.

The application of 8-Br-cADPR, which is a cADPR-antagonist, also inhibited the responses to cAMP-increasing odorant cocktails I and II (Figure 7). The magnitude of the responses to the IP_3-increasing odorant cocktail was not inhibited by 8-Br-cADPR. These results suggest that cADPR contributes to the generation of the responses to cAMP-increasing odorants.

Discussion

The present study supplies evidence for a physiological role for the candidate messenger cADPR in the turtle olfactory

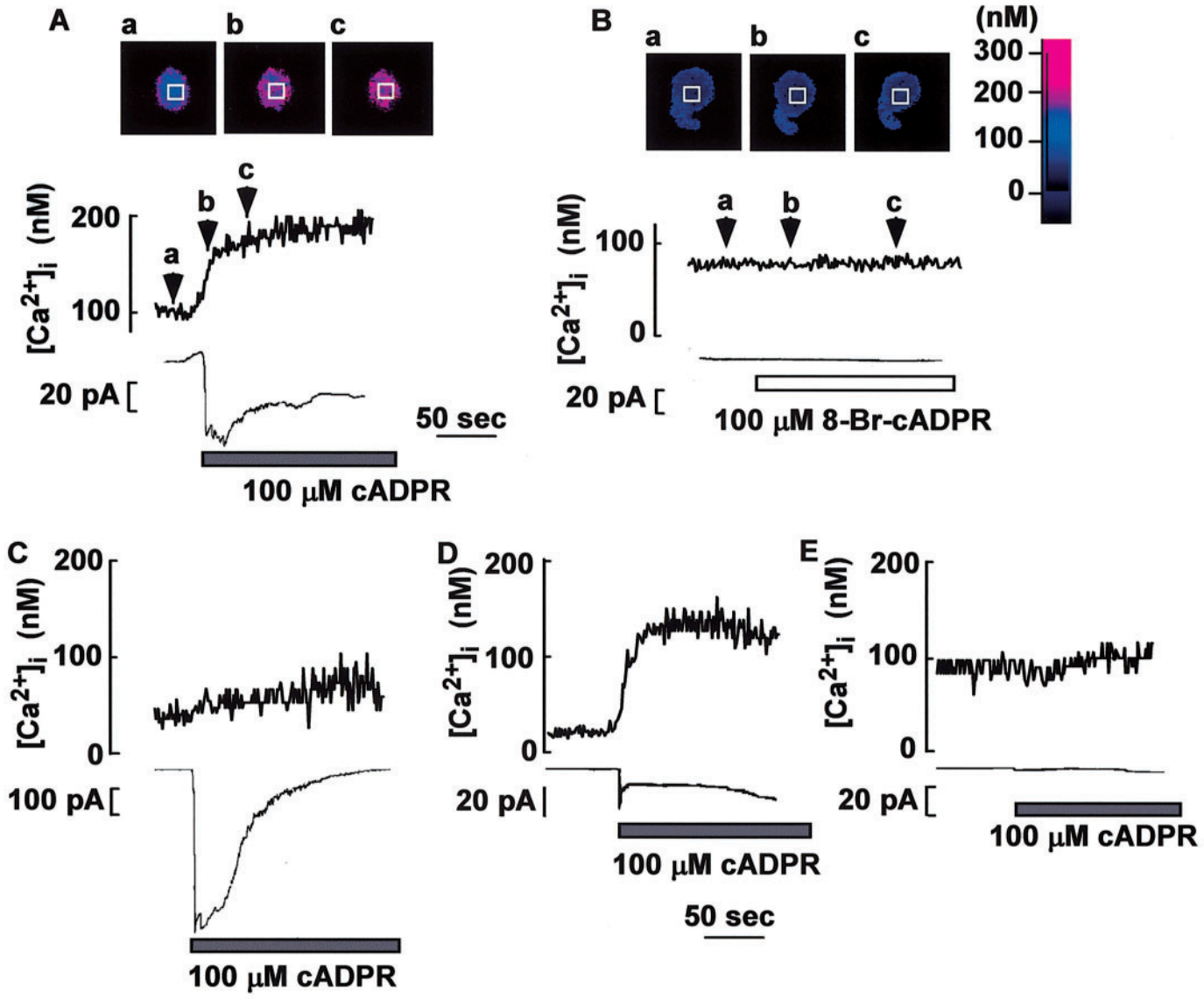

Figure 4 Changes in $[Ca^{2+}]_i$ and membrane current induced by 100 μM cADPR or and 100 μM 8-Br-cADPR in normal Ringer solution **(A, B)**, Ca^{2+}-free Ringer solution **(C)**, Na^+-free Ringer solution **(D)** and Na^+, Ca^{2+}-free Ringer solution **(E)**. Traces $[Ca^{2+}]_i$ and membrane currents were superimposed with a drawing soft, Canvas™ (Denabe System, Inc., Miami, FL). Color images were taken at a, b and c indicated in traces of $[Ca^{2+}]_i$. The bar in the lower trace indicates the period of cADPR stimulation.

transduction. We have shown that the dialysis of cADPR to olfactory receptor cells induces inward currents and increases $[Ca^{2+}]_i$ under the voltage-clamp conditions, and that the magnitudes of inward current responses to cAMP-increasing odorants is greatly reduced by inhibition of a cADPR-dependent pathway. However, we cannot entirely exclude the possibility that cADPR activates cAMP-gated ion channel directly or indirectly.

Voltage-gated Ca^{2+} channels exist at the somal membranes of olfactory cells (Frings *et al.*, 1991; Schild *et al.*, 1995). Depolarization induced by elevation of a K^+ concentration in the outer solution increased $[Ca^{2+}]_i$ without stimulation with odorants (data not shown). It is therefore theoretically possible that the increase in $[Ca^{2+}]_i$ caused by cADPR is due to the opening of olfactory transduction channels at the cilia, followed by the opening of voltage-gated Ca^{2+} channels at the soma. This possibility must be excluded, however, because a holding potential of –70 mV prevails at the somal membrane.

In general, cADPR induces Ca^{2+} release from endogenous Ca^{2+} stores in various types of cells (Berridge, 1993; Guse, 1999). In the olfactory cells, however, $[Ca^{2+}]_i$ remains low during a flow of inward currents induced by the dialysis of cADPR in a Ca^{2+}-free solution. This result indicates that Ca^{2+}-release from intracellular somal stores is not a primary source of the increase in $[Ca^{2+}]_i$ under these experimental

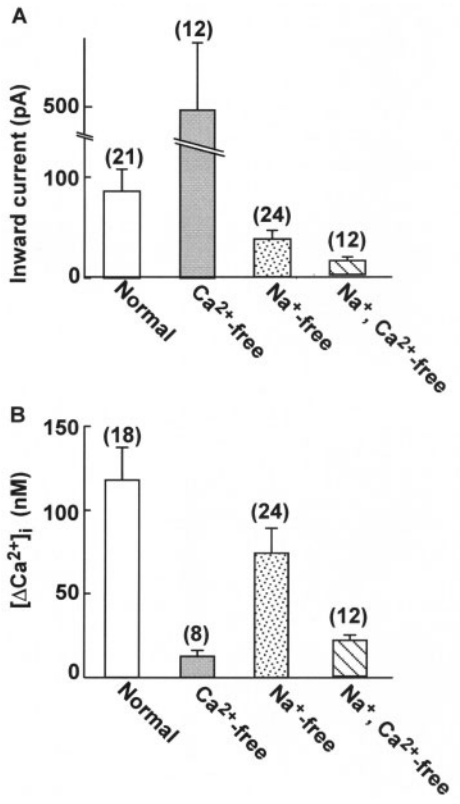

Figure 5 Mean peak values of inward currents **(A)** and $[Ca^{2+}]_i$ **(B)** recorded after the application of 100 μM cADPR while cells were exposed to Ringer solutions of different Na^+ and Ca^{2+} concentrations.

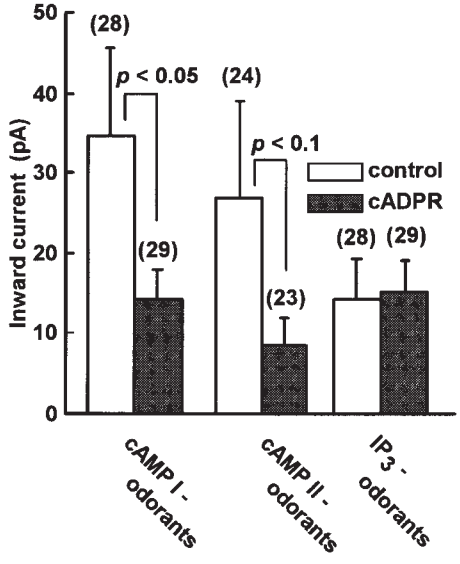

Figure 6 Inhibition of odor response by desensitization of responses to cADPR. Mean peak values of inward currents recorded by normal inner solution and inner solution containing 100 μM cADPR. cAMP-increasing odorant cocktail I; cAMP I-odorants, cAMP-increasing odorant cocktail II; cAMP II-odorants, IP_3-increasing odorant cocktail; IP_3-odorants. Vertical bars represent the mean ± SEM.

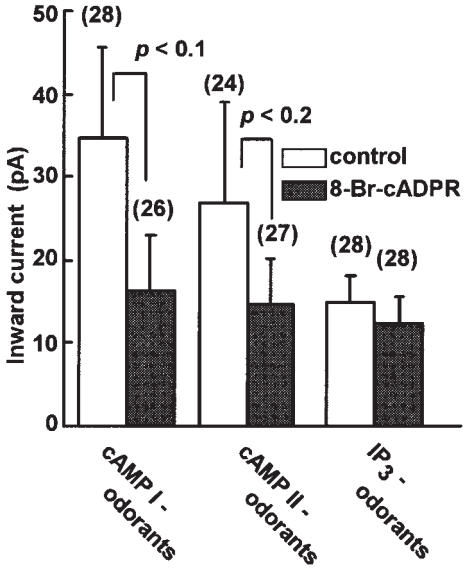

Figure 7 Inhibition of odor response by dialysis of 8-Br-cADPR. Mean peak values of inward currents recorded by normal inner solution and inner solution containing 100 μM 8-Br-cADPR. cAMP-increasing odorant cocktail I; cAMP I-odorants, cAMP-increasing odorant cocktail II; cAMP II-odorants, IP_3-increasing odorant cocktail; IP_3-odorants. Vertical bars represent the mean ± SEM.

conditions. In T-lymphocytes, the microinjection of cADPR dose-dependently induces repetitive Ca^{2+} spikes that are almost completely dependent on extracellular Ca^{2+}, indicating that cADPR induces Ca^{2+} entry into T cells (Guse *et al.*, 1997). The present results are in agreement with these observations.

The present results show that cADPR induces an inward current both in Ca^{2+}-containing and Ca^{2+}-free solutions. The magnitude of the inward currents in response to cADPR in Na^+-free Ringer solution is much less than that in normal Ringer solution, suggesting that the current is mainly carried by Na^+. These results suggest that cADPR activates a cation channel at the plasma membrane, allowing inflow of Na^+ and Ca^{2+} ions. The magnitude of inward current induced by cADPR in Ca^{2+}-free Ringer solution is larger than that in normal Ringer solution, suggesting that cADPR-induced currents are inhibited by the presence of extracellular Ca^{2+}.

Membrane-bound and soluble ADP-ribosyl cyclases catalyze the cyclization of NAD^+ to produce cADPR. The receptor-mediated formation of cADPR has been reported in intestinal longitudinal muscle cells (Kuemmerle and Makhlouf, 1995) and in NG 108-15 neural cells (Higashida *et al.*, 1997). Until now, the biochemical processes of formation of cADPR via receptors are not clear. The soluble enzymes from ascidian oocytes (Grumetto *et al.*, 1997) and phenochromocytoma (PC12) cells (Clementi *et al.*, 1996) are activated by NO via cGMP. The stimulation of rat olfactory cilia with a high dose of odorants elicits a delayed and

sustained elevation of cGMP, which is eliminated by an inhibitor of nitric oxide synthesis (Breer *et al.*, 1992) Therefore, it is possible that NO/cGMP participates in the formation of cADPR in response to odorants during sustained responses.

Receptors coupled with the accumulation of cADPR in response to odorants in olfactory cells remains to be investigated. Acetylcholine changes the activity of ADP-ribosyl cyclase in membranes of NG108-15 neuronal cells in a muscarinic receptor subtype-specific manner. The activation or inhibition of the cyclase activity is mimicked by GTP and blocked by cholera toxin, suggesting that G protein-coupled receptors modulate ADP-ribosyl cyclase via G proteins within cell membranes (Higashida *et al.*, 1997). Hence, it is possible that G protein-coupled receptors are linked to ADP-ribosyl cyclase. The binding of odorants to odorant receptors may lead to the activation of ADP-ribosyl cyclase via G proteins. This problem requires further experimentation.

Disruption of a cAMP-gated channel (Brunet *et al.*, 1996), G_{olf} (Belluscio *et al.*, 1998) or adenylyl cyclase III gene (Wong *et al.*, 2000) leads to anosmia in transgenic mice, suggesting that odor responses are generated entirely via cAMP-dependent pathways in the mouse. However, not all odorants activated adenylyl cyclase in olfactory cells of various animals (Sklar *et al.*, 1986; Breer and Boekhoff, 1991; Restrepo *et al.*, 1993a; Fabbri *et al.*, 1995; Okamoto *et al.*, 1996). Some of these odorants increased IP_3 concentrations in rat and sheep olfactory cilia preparations (Breer and Boekhoff; 1991; Fabbri *et al.*, 1995), suggesting that IP_3-dependent pathways may also play roles in the generation of odor responses. Application of cAMP-increasing odorants induced inward current responses in the sensory neurons of the *Xenopus* water nose, whereas dialysis of high concentration cAMP did not induce inward currents in these neurons (Iida and Kashiwayanagi, 1999, 2000). In addition, after complete desensitization of the cAMP-dependent pathway, various odorants induced large odor responses in these neurons (Kashiwayanagi *et al.*, 1994; Kashiwayanagi and Kurihara, 1995). These results suggest that odor responses to cAMP-increasing odorants are also generated via cAMP-independent transduction pathways.

Odorants, which increase cAMP concentration, have no effect on IP_3 concentration in rat and sheep olfactory cilia (Breer and Boekhoff; 1991; Fabbri *et al.*, 1995), suggesting that the cAMP-independent responses to cAMP-increasing odorants are not generated via IP_3. In the present study, we showed that the mean magnitude of the inward currents in response to 100 µM cADPR is comparable to or larger than that of the currents induced by odorants. The magnitudes of inward current responses to cAMP-increasing odorants are greatly reduced after desensitization of the cADPR-dependent pathway, which was achieved by previous dialysis of a high concentration of cADPR. The dialysis of 8-Br-cADPR, an antagonist, also inhibits responses to cAMP-increasing odorants. Therefore, it is likely that a part of the odor responses to the cAMP-increasing odorants is generated via a cADPR-dependent pathway.

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