Inositol 1,4,5-Trisphosphate-Gated Conductance in Isolated Rat Olfactory Neurons

YUKIO OKADA, JOHN H. TEETER, AND DIEGO RESTREPO
Monell Chemical Senses Center, and Department of Physiology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

SUMMARY AND CONCLUSIONS

1. The effect of intracellular application of inositol 1,4,5-trisphosphate (IP$_3$) from the patch pipette was analyzed in isolated rat olfactory neurons under whole-cell patch clamp.

2. Intracellular dialysis of 10 μM 1,4,5-IP$_3$ in K$^+$-internal solution induced a sustained depolarization of 35.8 ± 10.5 (SD) mV (n = 16). The IP$_3$-induced response was observed in 75% of the cells dialyzed with IP$_3$, but not when 10 μM ruthenium red was also included in the pipette solution (4 cells). Lower concentrations (50–100 nM) of 2,4,5-IP$_3$ induced similar responses to those produced by 1,4,5-IP$_3$ in five of eight olfactory neurons.

3. Steady-state I-V relationships of IP$_3$-gated currents with K$^+$-internal solution were classified into two types: outwardly rectifying and N-shaped in Ca$^{2+}$-internal solution outwardly rectifying and linear patterns were observed.

4. The IP$_3$-induced currents were inhibited by external Ca$^{2+}$ (1 mM). The reversal potentials of the Ca$^{2+}$-inhibitable currents were -16.1 mV (n = 2) and -29.0 ± 7.1 mV (n = 3) for the outwardly rectifying and N-shaped in Ca$^{2+}$-internal solution. The reversal potential was -5.9 ± 6.8 mV (n = 5) in the Ca$^{2+}$-internal solution.

5. In contrast, the Ca$^{2+}$-ionophore, ionomycin (5 μM) hyperpolarized the olfactory neurons and greatly potentiated the outward currents at positive holding membrane potential.

6. The data suggest that IP$_3$ can depolarize rat olfactory neurons without mediation by intracellular Ca$^{2+}$.

INTRODUCTION

Odorants interact with receptor proteins located in the cilia that extend from the dendrite of olfactory neurons, triggering a biochemical cascade that results in membrane depolarization and discharge of action potentials (Kinnamon and Getchell 1991; Lancet 1986). Previous biochemical studies (Huque and Bruch 1986; Pace et al. 1985; Sklar et al. 1986) and recent stopped flow experiments (Breer et al. 1990; Breer and Boekhoff 1991; Restrepo et al. 1993a) have shown that stimulation of isolated olfactory cilia with odorants results in increases in the level of either adenosine 3',5'-cyclic monophosphate (cAMP) or inositol 1,4,5-trisphosphate (IP$_3$). In addition, odor stimulation of olfactory neurons in culture increases both in IP$_3$ and cAMP (Ronnert et al. 1993). Odorants that stimulate cAMP formation trigger the opening of cAMP-gated cation channels in the ciliary membrane (Altenhofen et al. 1991; Dhallan et al. 1990; Frings et al. 1992; Kurahashi 1990; Lowe and Gold 1993; Nakamura and Gold 1987; Suzuki 1989; Zufall et al. 1991).

In catfish olfactory neurons, IP$_3$ levels increase rapidly upon stimulation with amino acid odorants (Huque and Bruch 1986; Restrepo et al. 1993a), and the increase in IP$_3$ concentration appears to activate a cationic conductance that has been shown to be different from that activated by cAMP (Miyamoto et al. 1992a; Restrepo et al. 1990). In addition, a similar IP$_3$-gated conductance has been characterized in lobster olfactory neurons in culture (Fadool and Ache 1992). However, it remains unclear how an odorant-induced increase in IP$_3$ concentration elicits a depolarization in mammalian olfactory neurons. To examine whether a mechanism similar to that in catfish olfactory neurons mediates IP$_3$-induced response in mammalian olfactory neurons, we characterized whole-cell currents elicited by intracellularly applied IP$_3$ in isolated rat olfactory neurons. The present experiments are consistent with the hypothesis that, for odorants that increase IP$_3$ concentration, receptor cell depolarization is mediated by direct activation of IP$_3$-gated channels.

METHODS

Preparation

Olfactory receptor neurons were dissociated from adult rats, which were euthanized by CO$_2$ inhalation. The olfactory epithelium was quickly removed and washed in Ca$^{2+}$-, Mg$^{2+}$-free saline containing 2 mM EDTA. The epithelium was cut into small pieces and incubated for 10 min in 3 ml of the same saline containing 5 mM L-cysteine and 15 U/ml papain (Sigma, St. Louis, MO). After removal of 2 ml of the dissociation solution, the tissue was gently triturated with a fire polished pipette. Dissociation was terminated by addition of 3 ml of normal saline solution containing 10 μg/ml leupeptin (Sigma). Olfactory receptor cells were readily distinguished by their characteristic bipolar morphology. The cells possessed a round soma 5–10 μm in diameter with a single dendrite 10–30 μm in length that terminated in a round olfactory knob. Immotile cilia could be seen extending from the olfactory knob of some of the neurons. Occasionally a piece of axon extending from the soma could be detected. The cells could be differentiated from respiratory cells that displayed a tuft of motile cilia and from sustentacular cells that did not possess a dendrite and olfactory knob.

Recording

Voltage- and current-clamp recordings were performed in the whole-cell configuration (Hamill et al. 1981) using an Axopatch 1B amplifier (Axon Instruments, Foster City, CA). Patch-pipettes were pulled from borosilicate glass capillaries (Kimble Kimax 51, Vineland, NJ) with a Sutter P-80/PC electrode puller (Sutter Instrument, San Rafael, CA). The tips of the electrodes were heat-polished with a microforge (Narishige MF-83, Tokyo, Japan) to a final resistance of 3-6 MΩ when filled with internal solution. Recordings were made from olfactory neurons that had settled on the
glass bottom of a chamber attached to the stage of an inverted microscope (Nikon Diaphot, Tokyo, Japan). The recording pipette was positioned with a hydraulic micromanipulator (Narashige MO-103N). The formation of g seals between the pipette and the cell surface was facilitated by applying weak suction to the interior of the pipette. After adjusting the holding voltage, the patch membrane was ruptured by application of brief voltage pulses and additional negative pressure. The current signal was low-pass filtered at 5 kHz, digitized at 125 kHz, acquired at a sampling rate of 0.25–10 kHz, and stored on an IBM-PC compatible personal computer running pCLAMP software (Axon Instrument), which was also used to control the D/A converter for generation of the clamp protocols. The indifferent electrode was a chlorided silver wire. The series resistance value after compensation was ~10 MΩ. Input resistance was calculated from the current generated by a 20 mV hyperpolarizing voltage step from the holding potential or by a 167 mV/s voltage ramp from -80 to -60 mV. Cell capacitance was read directly from the amplifier after electronic compensation of the capacitative transient.

Averages are given as mean ± SD. Significance of differences between means was assessed using the Student’s t test. A P value of 0.05 was considered to be statistically significant.

Solutions and drugs

Normal saline solution consisted of (in mM): 145 NaCl, 5 KCl, 1 CaCl₂, 1 MgCl₂, 1 Na-pyruvate, 20 Na-N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.2. For stock solutions, ruthenium red (1 mM: Serva, Westbury, NY) and CaCl₂ (200 mM) were dissolved in deionized water and ionomycin (2 mM; Calbiochem, La Jolla, CA) was dissolved in dimethyl sulfoxide. Aliquots of the stock solutions were added to normal saline solution to give the desired final concentrations. The normal pipette solution (K⁺-internal) contained in mM 135 KCl, 0.1 CaCl₂, 1 MgCl₂, 1 ethylene glycol-bis(β-aminooethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 10 K-HEPES, pH 7.1. In some experiments KC1 was replaced with CsCl (100 mM; Calbiochem and IC Services, Wohrhn, MA). 1.4,5-IP₃ (10 μM, Calbiochem), and ruthenium red (1 mM) were dissolved in deionized water and added to the pipette solutions to provide the appropriate final concentration.

All experiments were carried out at room temperature (20–25°C).

RESULTS

Resting and voltage-activated properties

With K⁺-internal solution in the pipette, rat olfactory neurons maintained resting potentials ranging from -25 to -75 mV (-48.4 ± 10.8, mean ± SD, n = 25). The input resistance ranged from 0.5 to 4.8 GΩ (2.1 ± 1.0 (SD) GΩ, n = 20), and the membrane capacitance averaged 3.5 ± 0.8 pF (n = 20).

Voltage-clamped olfactory neurons displayed transient inward currents followed by sustained outward currents in response to depolarizing voltage steps from a holding potential of -80 mV (Fig. 1A). Transient inward currents, which were observed in 5 of 22 cells, activated between -60 and -40 mV, reached a peak at about -20 mV and inactivated rapidly. These properties are similar to those measured in rat olfactory neurons in culture (Trombley and Westbrook 1991) but differ from measurements in acutely isolated rat olfactory neurons where activation of the sodium current took place at more negative potentials (Rajendra et al. 1992). Outward currents, which were observed in all olfactory neurons, activated between -50 and -20 mV and displayed only slight inactivation during a 40-ms step. The outward currents were blocked by addition of 5 mM Ba²⁺ to the bath or when Cs⁺ was substituted for K⁺ in the internal pipette solution. Although a detailed analysis of these outward currents was not done, the measurements were in general agreement with those previously described in rat olfactory receptor neurons (Lynch and Barry 1991; Trombley and Westbrook 1991). The transient inward currents were tentatively identified as sodium currents and the outward currents as potassium currents.

IP₃ induced depolarization

When the pipette was filled with K⁺-internal solution, the mean membrane potential immediately after membrane rupture was -42.1 ± 12.8 mV (n = 25). The cells then hyperpolarized quickly and attained a steady-state membrane potential within 5–10 min (Fig. 2A). The final steady state membrane potential was on the average 6.3 ± 8.4 mV below the membrane potential immediately after rupture of the cell membrane. When the pipette was filled with K⁺-internal solution containing 10 μM 1,4,5-IP₃, a sustained depolarization of 35.8 ± 10.5 mV (n = 16) from an average initial membrane potential of -27.1 ± 11.2 mV was observed (Fig. 2B). Responses to 5–10 μM 1,4,5-IP₃ were examined in 28 receptor cells under current or voltage clamp. Twenty one cells (75%) displayed membrane potential depolarization (Fig. 2B) or an increase in inward current (Fig. 2E) at negative holding potentials when IP₃ was present in the pipette. The onset of the response was quite fast, as indicated by the difference in the initial mem-
brane potential between control olfactory neurons 
\((-42.1 \pm 12.8 \text{ mV}, n = 25)\) and neurons dialyzed with
1,4,5-IP3 \((-27 \pm 11.2 \text{ mV}, n = 16)\). This made it impossible

to estimate response latency. The time to peak for the re-

sponse was highly variable (30 to 1,200 s). The initial in-

ward current component observed at negative potentials

disappeared near 0 mV and was followed by the develop-

ment of an outward current that was clearly visible at 0 mV

(Fig. 2E).

The IP3-induced depolarization was not inhibited by ad-

dition of ruthenium red (5–20 \text{ \mu M}) to the bath. This is in

contrast with IP3-induced responses in catfish olfactory neu-

rons that were blocked by bath applied ruthenium red (Re-

strepo et al. 1990). In rat olfactory neurons, external appli-

cation of ruthenium red induced further membrane depo-

larization and a marked decrease in membrane resistance

in 2 of 4 cells depolarized by IP3. In contrast, addition of 10

\text{ \mu M} internal ruthenium red, as well as 10 \text{ \mu M} 1,4,5-IP3 to

the pipette solution prevented the IP3-induced depolariza-

tion (the initial resting potential of \(-43.3 \pm 15.9 \text{ mV}\) was

followed by a hyperpolarization of \(-2.5 \pm 3.3 \text{ mV}, n = 4\),

consistent with the response in control cells (Fig. 2C). The nonhydrolyzable analogue 2,4,5-IP3 displays a high affinity

for the rat olfactory IP3 receptor in receptor binding assays

(Restrepo et al. 1992). In agreement with these data, dialy-

sis with 2,4,5-IP3 (50–100 nM) also induced a depolariza-

tion in 5 of 8 olfactory neurons. In two of these cells the

response to 2,4,5-IP3 was a sustained depolarization, while

in three other cells the depolarization was transient (Fig. 2D).

Voltage dependence of IP3-induced response

The voltage dependence of the IP3-induced currents was

examined by applying a voltage ramp from \(-100\) to 100

\text{ mV} (167 \text{ mV/s}) to voltage-clamped olfactory neurons dur-

ing the response produced by 10 \text{ \mu M} 1,4,5-IP3. The result-

ing quasi-steady state current-voltage curves were of two
general forms, those displaying moderate outward rectifica-
tion (Fig. 3A, 6 cells) and those showing a distinct plateau

or N-shape at positive voltages (Fig. 3 B, 8 cells). Both types
of IP3-induced currents were partially blocked by addition
of 1 mM Cd2+ to the bath solution (5 trials). The Cd2+ sen-
sitive component of the outwardly rectifying type averaged

\(-80 \text{ pA} (n = 2)\) at \(-60 \text{ mV}\) and had a mean reversal poten-
tial of \(-16.1 \text{ mV}\). The Cd2+-sensitive component of the

current displaying a plateau was N-shaped, averaged

\(-106.3 \pm 54.9 \text{ pA} (n = 3)\) at \(-60 \text{ mV}\), and reversed at

\(-29.0 \pm 7.1 \text{ mV}\). Internal dialysis of a lower concentration

of 2,4,5-IP3 (100 nM) elicited a current with similar charac-
teristics to those evoked by 10 \text{ \mu M} 1,4,5-IP3 (Fig. 4). The current produced by 2,4,5-IP3 was also suppressed by Cd2+

and the Cd2+-sensitive component of the current was N-

shaped.

In control cells (without internal IP3 dialysis) the current
elicited by voltage ramps showed only slight outward rectification at potential more positive than 70 mV and never displayed the N-shaped I-V relationship shown by ~60% of the cells that responded to IP$_3$ (of Fig. 3A with 5B). In addition, although external Cd$^{2+}$ suppressed the whole-cell current by 60–70% in control cells (Fig. 5A), the Cd$^{2+}$-sensitive component in control cells (no IP$_3$ dialysis) was small and positive (<10 pA at -60 mV), and reversed near -80 mV, compared with the values between -40 and -10 mV for the Cd$^{2+}$-sensitive component of the IP$_3$-induced currents (Figs. 3 and 4).

The region of negative slope of the IP$_3$-induced current shown in Fig. 3B is typical of currents that include a contribution from Ca$^{2+}$-activated K$^+$ channels. To test this possibility, we examined the whole-cell I-V relationship in cells whose cytoplasm was dialyzed with 10 μM 1,4,5-IP$_3$ in pseudointracellular medium with K$^+$ replaced by Cs$^+$. The internal cation replacement should inhibit Ca$^{2+}$-activated K$^+$ channels but not the IP$_3$-gated channels, which should be permeable to Cs$^+$. Under these conditions, the I-V relationship of the IP$_3$-induced current was nearly linear (Fig. 6, 6 cells) or slightly outwardly rectifying (4 cells). An N-shaped component was not observed in the presence of internal Cs$^+$. The IP$_3$-induced current was inhibited by external Cd$^{2+}$ (1 mM) in five trials, and the mean reversal potential of the Cd$^{2+}$-inhibitable component in the presence of internal Cs$^+$ was 5.9 ± 6.8 mV (n = 5). These observations show that the N-shaped currents observed in 60% of the cells that were stimulated with internal IP$_3$ in K$^+$-internal solution (Fig. 3B) resulted from contributions of at least two different types of currents: a Ca$^{2+}$-activated K$^+$ current that is inhibited by replacement of K$^+$ by Cs$^+$, and a Cs$^+$-insensitive current that has an I-V relationship consistent with a nonspecific cation conductance (Fig. 6).

### Ionomycin-induced response

It is well established that IP$_3$ elicits release of Ca$^{2+}$ from intracellular stores in response to a variety of hormones and neurotransmitters (Berridge 1993; Ferris and Snyder 1992). In a similar manner, the responses of rat olfactory neurons to internal IP$_3$ could be mediated by an IP$_3$-induced increase in cytosolic Ca$^{2+}$, caused by release of Ca$^{2+}$ from internal stores. To examine this possibility, we determined the effect of increases in intracellular Ca$^{2+}$, elicited by addition of the Ca$^{2+}$-ionophore ionomycin, on whole-cell currents in rat olfactory neurons. When neurons dialyzed with K$^+$-internal solution were exposed to 5 μM ionomycin, the mean resting potential increased from -42.2 ± 14.1 mV (n = 6) to -85.0 ± 16.3 mV (Fig. 7A) and the
**Discussion**

The present study demonstrates that rat olfactory neurons respond to intracellular dialysis of IP₃ with an increase in inward current at negative membrane potentials. This response occurred at micromolar 1,4,5-IP₃ and nanomolar 2,4,5-IP₃ concentrations and was inhibited by intracellular ruthenium red and extracellular cadmium. In rat olfactory neurons, IP₃ induced a depolarization accompanied by an increase in conductance (Figs. 2 and 3), while the Ca²⁺-ionophore ionomycin elicited a hyperpolarization (Fig. 7). This suggests that the IP₃-induced depolarization in rat olfactory neurons is not mediated by increases in intracellular Ca²⁺. The I-V relationship displayed by the IP₃-induced conductance (Figs. 3 and 6) is consistent with the opening of plasma membrane IP₃-gated cation channels slightly selective for Ca²⁺ as postulated by us based on measurement of IP₃ binding to isolated rat olfactory cilia and of IP₃-gated currents in phospholipid bilayers into which cilia membrane vesicles had been incorporated (Restrepo et al. 1992). However, these results do not rule out the possibility that the IP₃-induced depolarization could include a component resulting from a localized increase in Ca²⁺ causing opening of a ciliary Ca²⁺-dependent Cl⁻ conductance (Kleene 1993, Kleene and Gesteland 1991, Kurahashi and Yau 1993).

In previous studies, a Ca²⁺-activated K⁺ conductance was not demonstrated in rat olfactory neurons (Lynch and Barry 1991, Trombley and Westbrook 1991), while olfactory neurons from other species have been shown to possess this conductance (Firestein and Werblin 1987; Maue and Dionne 1987, McClintock and Ache 1989; Miyamoto et al. 1992b; Schild 1989). In the present experiments, we observed only slight outward rectification in the I-V relationship elicited by voltage ramps in control cells (about 26 nM internal Ca²⁺ concentration). However, approximately 60% of the cells examined displayed an N-shaped I-V relationship characteristic of Ca²⁺-activated K⁺ currents after IP₃ dialysis or addition of ionomycin. The intracellular Ca²⁺ concentration in rat olfactory neurons increased from ~30 nM at rest to ~150 nM after application of 5 μM ionomycin (Restrepo et al. 1993). Therefore, the data in...
Fig. 6. Effect of replacement of internal K⁺ with Cs⁺ on whole cell I–V relationship for current evoked by internal 10 μM 1,4,5-IP₃. The current was induced by a voltage ramp (167 mV/s) from −100 to 100 mV. The pipette contained Cs⁺-internal solution with 1,4,5-IP₃. A: addition of 1 mM Cd²⁺ to external solution inhibited the 1,4,5-IP₃-induced current. B: Cd²⁺-sensitive current was obtained by subtracting (a) from (b). The reversal potential was −12.7 mV.

Fig. 7 indicate that Ca²⁺-activated K⁺ channels in rat olfactory neurons appear to be activated by Ca²⁺ concentrations over 100 nM. This interpretation is consistent with the Ca²⁺ sensitivity of the Ca²⁺-activated K⁺ conductance in mouse olfactory neurons (Maue and Dionne 1987).

The IP₃-induced depolarization in olfactory neurons from catfish (Miyamoto et al. 1992a; Restrepo et al. 1990) and lobster (Fadool and Ache 1992) was transient, in contrast to the sustained response observed in most rat olfactory neurons in the present study. The transient response to sustained dialysis of IP₃ in catfish olfactory neurons was attributed in part to opening of Ca²⁺-activated K⁺ channels, which would tend to repolarize the cells. Some rat olfactory neurons displayed N-shaped outward currents (Fig. 3B), which were similar in shape, but not in reversal potential to those produced by ionomycin (Fig. 7), and the N-shaped component of the IP₃-induced current was abolished by replacement of intracellular K⁺ by Cs⁺. Furthermore, the reversal potential of the rat olfactory IP₃-gated conductance in lipid bilayers was about −5 mV, while that of the whole-cell preparation in K⁺-internal solution was more negative (about −24 mV), suggesting that the current induced by intracellular dialysis of IP₃ includes a K⁺ component. These observations indicate that an increase in the intracellular concentration of Ca²⁺, presumably mediated by IP₃-gated channels, activates a Ca²⁺-sensitive K⁺ conductance in some rat olfactory neurons as well.

Under conditions of internal IP₃ dialysis, the membrane potential would be determined by a balance of the IP₃-gated conductance and Ca²⁺-activated K⁺ conductances. In rat olfactory neurons, the steady-state contribution of the IP₃-gated conductance to the membrane potential is larger than that of the Ca²⁺-activated K⁺ conductance thereby leading to a steady depolarization (Fig. 3). The difference in the time course of the IP₃-response under current clamp between catfish and rat olfactory neurons could be explained by a larger relative contribution from a Ca²⁺-activated K⁺ conductance to the IP₃-induced current in catfish. Development of a large efflux of K⁺ through the Ca²⁺-activated conductance in catfish would cause the membrane potential to hyperpolarize giving rise to a transient response. Simi-
IP$_3$-INDUCED CURRENT IN RAT OLFACTORY NEURONS

larly, the more potent nonhydrolyzable analogue 2,4,5-IP$_3$ could produce a transient response in 50% of rat olfactory neurons under current clamp (Fig. 2 D) because it would be expected to exert a larger, more prolonged effect than 1,4,5-IP$_3$ thereby leading to an influx of Ca$^{2+}$ large enough to cause hyperpolarization mediated by opening of Ca$^{2+}$-activated K$^+$ channels.

In isolated rat olfactory cilia membranes (Breer et al. 1990; Breer and Boekhoff 1991), and in rat olfactory neurons in culture (Ronnett et al. 1993), some odorants can stimulate rapid formation of IP$_3$. In addition, rat olfactory cilia have been shown to possess an IP$_3$ receptor channel protein (Restrepo et al. 1992) and preliminary immunohistochemical evidence with anti-IP$_3$-receptor antibodies indicates that IP$_3$ receptors are localized to the cilia and dendrites of rat olfactory neurons (Cunningham et al. 1991; Kalinoski et al. 1993). The results of the present study suggest that odorants that elicit an increase in IP$_3$ concentration in rat olfactory cilia ultimately activate an IP$_3$-gated conductance, leading to membrane depolarization and action potential discharge. Furthermore, our experiments also indicate that the opening of the IP$_3$-gated channels leads to an influx of Ca$^{2+}$ eliciting an increase in [Ca$^2+$. The increase in Ca$^{2+}$ concentration, which presumably takes place faster in the lumen of the olfactory cilia, could cause opening of Ca$^{2+}$-activated Cl$^-$ channels thereby contributing to cell depolarization (Kleene 1993; Kurahashi and Yau 1993). In addition, in rat olfactory neurons [Ca$^2+$ also increases, albeit more slowly, in the olfactory knob and dendrite, and in some cells in the cell body (Restrepo et al. 1993), where it is expected to cause opening of Ca$^{2+}$-activated K$^+$ channels (Maue and Dione 1987) thereby leading to repolarization of the cell membrane. Therefore it appears that, as postulated for catfish (Restrepo et al. 1990; Kalinoski et al. 1992) and lobster (Fadool and Ache 1992) olfactory neurons, mositol-1,4,5-triphosphate plays a mediatory role in olfactory transduction in mammals through regulation of an IP$_3$-sensitive cation conductance.

This work was supported by National Institute of Deafness and Other Communications Disorders Grant DC-00566. Address for reprint requests: D. Restrepo, Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104-3308.

Received 1 July 1993; accepted in final form 20 October 1993

REFERENCES


