*Journal of Bioenergetics and Biomembranes, Vol. 20, No. 5, 1988*

# MINI-REVIEW

# Ion Channels from Chemosensory Olfactory Neurons

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*Received May 24, 1988*


## Abstract

The olfactory epithelium has the ability to respond to a large number of volatile compounds of small molecular weight. Ultimately, such a property lies on a specialized type of neuron, the olfactory receptor cell. In the presence of odorants, the olfactory receptor neuron responds with action potentials whose frequency depends on odorant concentration. The primary events in the process of olfactory transduction are thought to occur at the cilia of olfactory receptor neurons and involve the binding of odorants to receptor molecules followed by the opening of ion channels. A crucial step in understanding olfactory transduction requires identifying the mechanisms that regulate the electrical activity of olfactory cells. In the last couple of years, patch-clamp recording from isolated olfactory cells and reconstitution of olfactory membranes in planar lipid bilayers have begun to shed light on some of these mechanisms. Although the information emerging from such studies is still preliminary, there are already well-defined hypotheses on the molecular events that might underlie the primary events in olfactory transduction. Currently, attention is being focused on the notions that second messengers might be involved in the activation of ion channels in olfactory cilia, and that odorant binding to a receptor molecule might lead directly to the gating of ion channels in chemosensory olfactory membranes. The coming years promise to be exciting ones in the field of olfactory transduction. We have now the necessary tools to be able to confront hypotheses and experimental facts.


**Key Words:** Olfactory transduction; olfactory receptor neurons; ion channels.

## Introduction

The primary events involved in odorant transduction occur at chemosensory membranes of olfactory receptor neurons located in the olfactory epithelium.

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0145-479X/88/1000-0551$06.00/0 

Olfactory receptors are present in small (5- to 10-$\mu$m diameter) bipolar cells projecting a 50 to 100-$\mu$m-long dendrite toward the epithelial surface. The dendrite ends in a "dendritic knob" on which 5–20 olfactory cilia are anchored. The cilia are immersed in a mucus layer 30–50 $\mu$m thick. Odorants entering through the nasal cavity dissolve in the mucus and reach the olfactory cilia where, according to the available experimental evidence, the primary events in olfactory transduction take place (Getchell, 1986; Lancet, 1986; Anholt, 1987). Odorant binding to receptors in the olfactory ciliary membrane results in a depolarization that triggers action potentials in the soma of olfactory cells. The regenerative electrical activity propagates to the unmyelinated axons of primary olfactory receptor neurons. Hundreds of these asons form bundles that merge to form the olfactory nerve. The axons project to the olfactory bulb where 100–1000 of them synapse with a single mitral cell, giving rise to synaptic structures known as glomeruli (Shepherd, 1972; Shepherd, 1983). The olfactory bulb is where the first steps in the integration of olfactory information take place.

In addition to chemosensory olfactory neurons, the olfactory mucosa is made of sustentacular cells that extend microvilli processes to the epithelial surface and that might have a secretory role (Okano and Takagi, 1974; Getchell, 1986). A third type of cell, the basal cells, lie deep in the mucosa and undergo constant division and differentiation to produce mature olfactory neurons (Fig. 1A). The olfactory epithelium is peculiar in that it contains the only population of neurons under constant renewal and the only place in the nervous system in which neurons are exposed directly to the outer environment.

Electrophysiologically, olfactory receptor cells can be distinguished from basal and supporting cells because of their ability to fire action potentials in response to depolarizations or in response to odorants (Suzuki, 1982; MacLeod and Trotier, 1983; Masukawa *et al.*, 1985). On the other hand, both olfactory neurons and basal cells share in common the feature of having a high input resistance (Suzuki, 1982; Masukawa *et al.*, 1985; Trotier and MacLeod, 1983; Trotier, 1986). The high input resistance of olfactory cells ($>6$ gigaohms) implies that opening of only one or two ion channels having conductances equivalent to the ion channel of the acetylcholine receptor (30–50 pS) in the dendrite or soma would be enough to induce the firing of an action potential (Fenwick *et al.*, 1982; Trotier, 1986). The resting potentials of olfactory and basal cells rise between $-60$ and $-30$ mV (Suzuki, 1982; Masukawa *et al.*, 1985; Trotier and MacLeod, 1983; Trotier, 1986). In contrast, sustentacular cells have low input resistances and exhibit more negative resting potentials with values ranging between $-120$ and $-80$ mV (Masukawa *et al.*, 1985).

Because of their small dimensions, olfactory neurons are hard to impale with microelectrodes. However, studies based on extra- and intracellular

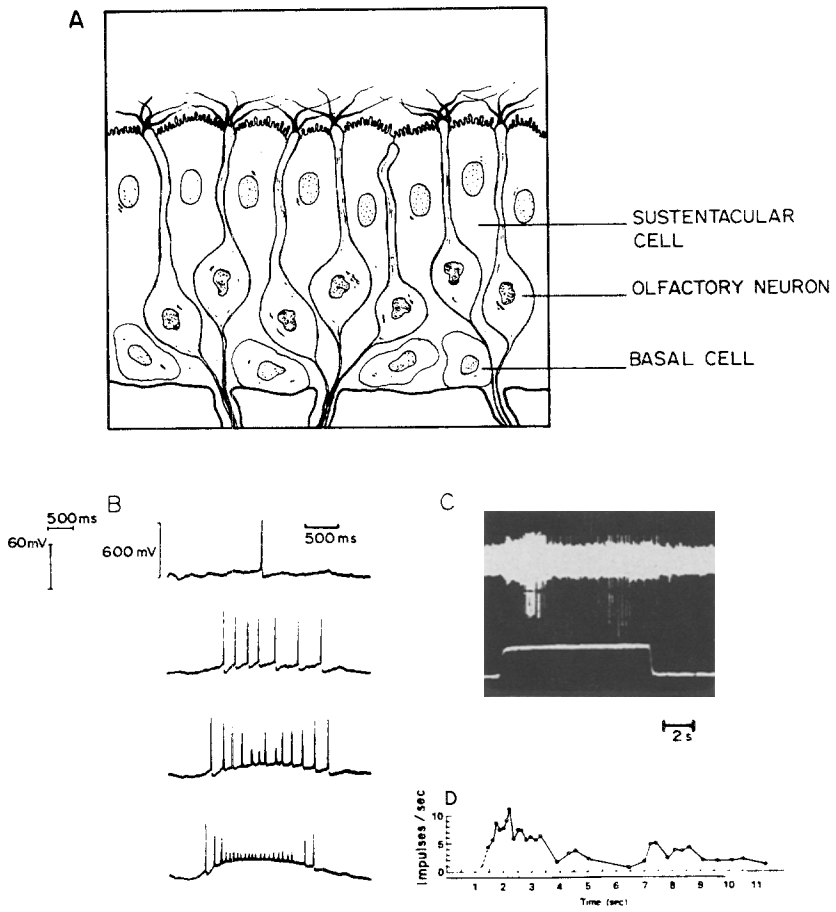


**Fig. 1.** (A) Drawing of the olfactory epithelium showing the three types of cells that can be found in it: olfactory receptor neurons, sustentacular cells, and basal cells. (B) Four responses to puffs of odorants at different concentrations increasing toward the bottom of the Fig. The membrane potential was recorded by impaling a receptor neuron with an intracellular microelectrode. Modified from Trotier and MacLeod (1983). (C) Adaptation of an olfactory receptor to a prolonged odorant stimulus. The extracellular recording shows how the frequency of firing decays during stimulation. Bottom trace: stimulus monitor. Modified from Getchell and Shepherd (1978a). (D) Plot of the firing frequency (impulses per second) versus time shows the decay of firing frequency along the response shown in C. Modified from Getchell and Shepherd (1978a).

recordings have unveiled some of their electrical properties, in particular those related to odorant responses. These studies indicate that each receptor cell responds to several, but not all, odorants (Baylin, 1979; Baylin and Moulton, 1979; Getchell, 1986; O'Connell and Mozell, 1968; Sicard and Holley, 1982; Trotier and MacLeod, 1983). Odorant concentration determines the rate of action potential firing. Upon application of a prolonged

puff of odorant, the cell undergoes a polarization that develops slowly and is accompanied by action potentials whose rate is proportional to odorant concentration. At the plateau level of the odorant-induced depolarization, the amplitude of the spikes decreases. Firing stops during the repolarizing phase of the odorant-induced slow response, even before the membrane potential reaches a subthreshold value (Fig. 1B). The input resistance greatly decreases during the response of the cell to the odorant (Baylin, 1979; Getchell, 1986; Getchell and Shepherd, 1978a; Trotier and MacLeod, 1983). Long exposures to odorants result in an initially high rate of firing that decays with time to a low, steady firing rate. This behaviour might be the basis for the phenomenon of odor adaptation (Fig. 2C) (Baylin and Moulton, 1979; Getchell, 1986; Getchell and Shepherd, 1978b; Ottoson, 1956).

Current patch-clamp studies of intact, isolated olfactory neurons and reconstitution studies in planar lipid bilayers of chemosensory membranes derived from olfactory cilia are beginning to shed light on the mechanisms underlying the process of olfactory transduction in the cilia as well as the regulation of electrical activity in the dendrite and soma of olfactory receptor neurons.

## Isolation of Vertebrate Olfactory Receptor Neurons

Patch-clamp studies have been performed on isolated olfactory receptor neurons of vertebrates. Several procedures have been developed to dissociate and isolate these cells from the olfactory mucosa. Dissociation and isolation procedures are crucial to obtain healthy receptors. The most obvious criterion to discriminate between healthy and unhealthy isolated cells is their ability to respond to odorants. Although direct electrophysiological evidence demonstrating odorant responses in isolated vertebrate olfactory cells is scarce, all reported isolation procedures seem to yield morphologically healthy-looking cells displaying cilia in their dendritic knobs and ciliary beating. Receptor cells have been isolated from salamander, mouse, pig, and frog olfactory epithelia.

Maue and Dionne (1987) combined trypsin treatment, divalent free buffer solution, and mechanical disruption to obtain large numbers of olfactory receptors from mice. According to these authors, the isolated receptors obtained following their procedure maintained intact their characteristic morphology and displayed dendritic knobs bearing as many as 20 cilia. In patch-clamp studies, Maue and Dionne found that a fraction of isolated receptors were responsive to a mixture of odorants.

A two-step procedure to isolate olfactory cells from porcine olfactory mucosa has been developed by Kashiwanayagi *et al.* (1987). The first step

involved cell dispersion by gentle pipetting. Integrity of the cells was estimated to be ~ 50%, based on their ability to exclude nigrosin. In the second step, the suspension of dispersed cells was subjected to fractionation in a bovine serum albumin density gradient. Most of the cells grouped in the 1% fraction of the gradient. Cell integrity in this second step was also 50%. Porcine olfactory cells isolated by this procedure showed normal morphologic features, including cilia preservation. Responses to several odorants were monitored indirectly using the voltage-sensitive dye Rh6G.

Nakamura and Gold (1987) obtained olfactory cells from the olfactory mucosa of the frog *Bufo marinus* by enzymatic dissociation with papain, a

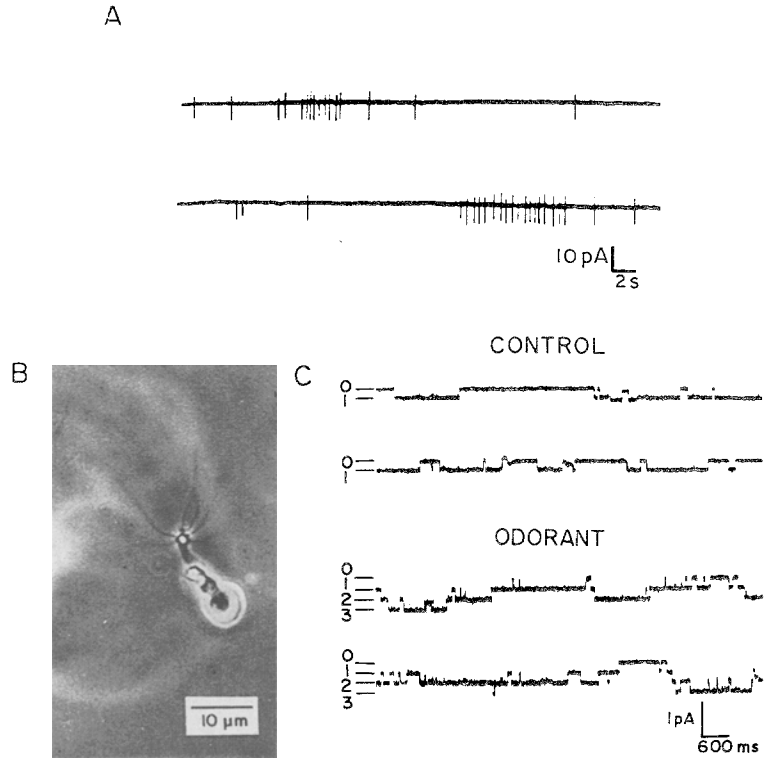


**Fig. 2.** Spontaneous firing of action potentials from an isolated frog olfactory neuron, as recorded with a cell-attached patch pipette (inward currents upward). (B) Phase-contrast micrograph of a dissociated frog olfactory receptor. (C) Channel activity recorded with a patch pipette in the cell-attached configuration (outward currents are shown downward). The top two traces are continuous recording of the patch-clamp current while the membrane potential inside the pipette was −70 mV and the bath contained control (Ringer solution). The two traces at the bottom are also continuous. They were obtained at the same potential, but after exposing the cell to a mixture of the bell pepper odorant galvanize and the citrus odorant citralva. Odorant addition caused an increase in channel activity, as shown in the Fig.

procedure that yielded morphologically normal olfactory cells. An alternative approach avoiding enzyme treatment or density gradients was developed by Trotier (1986), who used receptor cells from salamander olfactory epithelium. The procedure consists in scraping the mucosa with a microscalpel. The resulting cell aggregates are then passed in and out through a fire-polished Pasteur pipette followed by slow centrifugation (50 *g*, 5 min) and resuspension. Cells isolated by this procedure are responsive to odorants. Gigaohm seals are not easy to obtain in freshly dissociated cells. However, as reported by Trotier, seals are readily achieved 1 day after dissociation. At that time, cells have lost their peculiar morphology, becoming rounded and exhibiting small dendritic processes, but still maintaining ciliary beating. A slightly different procedure from that used by Trotier has been developed in our laboratory to isolate receptor cells from the olfactory epithelium of the frog *Caudiverbera caudiverbera*. After its removal from the nasal cavity, the epithelium is cut into small pieces with a razor blade. The finely cut pieces are then passed through a fire-polished pipette and subjected to slow centrifugation. Cells obtained by this procedure retain their morphology, display cilia and ciliary beating (Fig. 2A), and can be immediately used for patch-clamp studies yielding gigaohm seals in 25–30% of the trials (J. Bacigalupo and P. Labarca, unpublished). By means of a patch clamp, we have recorded a variety of channels from receptors isolated following our procedure. In addition, these cells display spontaneous electrical activity (Fig. 2B) and chemosensitivity reflected in changes in channel activity upon exposure to odorous agents (Fig. 2C).

## Patch-Clamp Studies in Isolated Olfactory Receptor Cells

### *Patch-Clamp Recording from Olfactory Cilia Membranes*

Use of different modalities of the patch-clamp technique has made it possible to obtain valuable information concerning the nature of the conductances present in ciliary, dendritic, and somatic membranes of isolated olfactory receptor neurons. So far, the most impressive experimental results are those reported by Nakamura and Gold (1987), who recorded from membrane patches excised from olfactory cilia of the frog. First, they demonstrated that the ciliary membrane is amenable to direct electrophysiological recordings. Their results showed that cilia, dendrites, and cell bodies of isolated olfactory cells possess a cation-selective conductance pathway that can be directly and reversibly activated by micromolar concentrations of cGMP and cAMP (Fig. 3A). The presence of this conductance in olfactory cells strengthens the idea, derived from biochemical studies of olfactory cilia (Pace *et al.*, 1985; Sklar *et al.*, 1986; Lancet, 1986), that second messengers

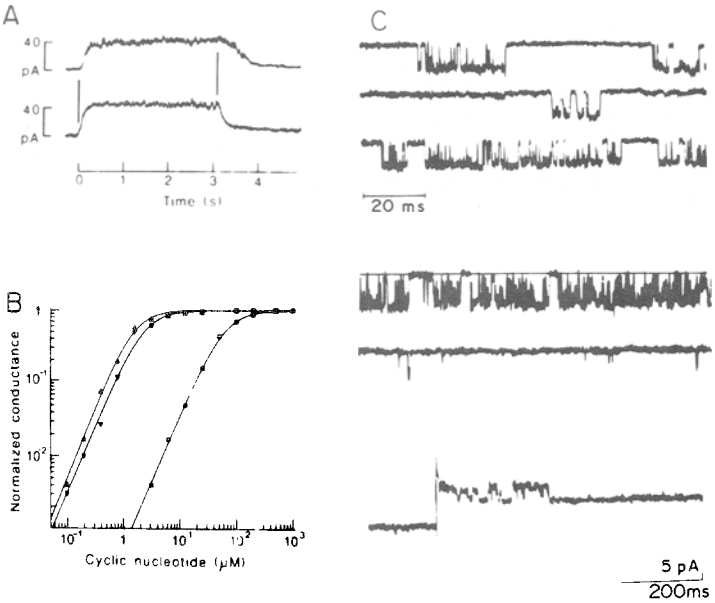


**Fig. 3.** Patch-clamp studies on olfactory receptors. (A) Inside-out, excised patch from ciliary membrane of an olfactory receptor showing, in the top trace, the development of an outward current as the bath is perfused with a solution containing 10 $\mu$m cAMP. Vertical bars indicate the time of perfusion with this solution. A similar result was obtained in the experiment shown in the bottom trace, in which a bath solution containing no $Na^+$ was replaced by another solution containing 110 mM $Na^+$; both solutions contained 10 $\mu$M cAMp. The voltage inside the pipette was 30 mV. (B) Plot showing concentration dependence of the cylic nucleotide-gated conductance. First curve from the left, cGMP; second curve, CAMP; and third curve, cGMP. A and B were modified from Nakamura and Gold (1987). (C) Single-channel record from olfactory receptors showing the activity of three different channels. Top three traces: 130-pS $Ca^{2+}$-activated $K^+$ channel. Two traces at the center: 80-pS $Ca^{2+}$-activated $K^+$ channel. Bottom trace: 40-pS $K^+$ channel. Modified from Maue and Dionne (1987).

might be involved in olfactory tranduction. The cationic channel activated by cyclic nucleotides does not select between sodium and potassium ions, suggesting that its activation leads to a depolarization that might play an excitatory role. The relative affinities of the cyclic nucleotide-gated conductance indicate that cGMP is a more potent activator than cAMP, and that cCMP is also effective as an activator. Relative affinities for cGMP range from 0.7 to 20 $\mu$M and from 2.4 to 37 $\mu$M for cAMP with Hill coefficients larger than unity, suggesting a cooperative effect. The scatter in apparent affinity values reported in these studies was interpreted as reflecting the occurrence of different receptor classes or different stages in the development of the receptors from which the records were obtained.

The current–voltage relation of the cyclic nucleotide-activated conductance is nearly ohmic at negative membrane potentials (patch pipette positive) and displays rectification at positive voltages. Rectification is abolished when divalent cations are not present, suggesting a voltage-dependent channel-blocking effect of these cations. Activation by cyclic nucleotides was reported to occur in a total of 46 excised patches. Since all patches reported showed this conductance, it might be concluded that it is a general feature of olfactory cilia and that it occurs in cells diplaying different degrees of development. Single-channel studies of ciliary membranes revealed that cyclic nucleotide-gated channels have a conductance of 50 pS (Nakamura and Gold, 1987). Nakamura and Gold did not report the presence of other types of channels in membrane patches excised from olfactory cilia.

From the results published by Nakamura and Gold (1987), one is tempted to estimate the density of cyclic nucleotide-gated channels in ciliary membranes. In the absence of divalent cations, which seem to block these channels, conductances of 1.3 and 8.0 nS were activated in excised patches by 10 $\mu$M cAMP and 3 $\mu$M cGMP, respectively. For simplicity, we shall assume that, at these concentrations of cyclic nucleotides, all channels are in the open state, an assumption that gives a lower limit for the channel density. The area of the membrane patch can be estimated considering that the cilia have diameters of 0.25 $\mu$m. Since the patch pipette should have, at most, the same diameter, the area of the excised membrane would be $5 \times 10^{-2}\,\mu m^2$. If, as reported (Nakamura and Gold, 1988), the channels have conductances of 30 pS, the channel density would be 800–5000 $\mu m^2$. These numbers can be compared with estimates of intramembrane particle density in ciliary membranes reported by Menco (1980), whose values range from 800 to 2500 particles per square micron. If intramembrane particles represent channels, the cyclic nucleotide-gated ones would represent a very large fraction of them in ciliary membranes.

### *Whole-Cell Recording*

The only reported study in isolated vertebrate olfactory cells using the whole-cell-recording modality of the patch-clamp technique is that of Trotier (1986) using isolated receptors from *Salamander salamander*. These studies provided estimates of input resistance in olfactory cells yielding values on the order of several gigaohms—much higher than those obtained previously using intracellular microelectrodes. Resting potentials measured by Trottier (1986) averaged −39 mV, and cell capacitance values of 6–11 pF were found for cells with diameters in the 14- to 18-$\mu$m range. Depolarization of receptor cells in the absence of odorants and under normal ionic conditions triggered a brief inward current followed by a long-lasting outward current. The

inward current seems to inactivate within 10–15 ms while the outward current displays a slower and incomplete inactivation. At its maximum, the inward current had a magnitude of 80–500 pA. Activation of this current occurs between $-20$ and $-30$ mV from holding potentials of $-50$ to $-60$ mV, reaching a maximum near 0 mV. Inward currents recorded in sodium-free saline containing 15 $\mu$M tetrodotoxin (TTX) and 1 mM calcium are greatly diminished but not abolished, yielding values of $\sim 10$ pA at $+10$ mV. Under these conditions, raising the calcium concentration to 10 mM results in a significant inward current that is suppressed by 10 mM $Co^{2+}$. Thus, calcium currents, although present under physiological conditions, make only a small contribution to the total inward current. On the other hand, inward currents measured in physiological saline but in the presence of 15 $\mu$M TTX look normal, but are blocked by millimolar concentrations of $Co^{2+}$. Trotier (1986) interpreted these experimental observations as evidence for the involvement of $Ca^{2+}$ channels in the inward current. Under normal conditions, in the presence of 1 mM $Ca^{2+}$ and 120 mM $Na^{+}$, sodium ions would permeate through these channels, making up most of the inward component.

Since the inward current displays fast inactivation, the outward potassium current is relatively easy to study under voltage clamp. This current activates between $-20$ and $-5$ mV from holding potentials of $-50$ mV, has a maximal amplitude of 200–600 pA for a depolarization to $+50$ mV, and displays an incomplete inactivation. Replacing potassium by $Cs^{+}$ and tetraethylammonium (TEA) in the patch pipette abolishes the outward current. The outward current is made of two components: an early one that inactivates within 200–300 ms and that is absent when the holding potential is set near the cell's resting potential, and a late component that activates at depolarizing voltages and that undergoes a slow and incomplete inactivation, with a time course of seconds.

### *Odorant-Activated Currents in Isolated Receptors*

Odorant-activated currents have been difficult to record from olfactory neurons because of the short lifetime of the preparation under whole-cell-recording conditions. Trotier (1986) has reported one case of a cell that responded several times to repetitive exposures to a mixture of 7 $\mu$M isoamylacetate and 10 $\mu$M butanol. The current activated by odorants is ohmic in the explored voltage range, having a slope conductance of 3.2 nS and a reversal potential near 0 mV. The current irreversibly disappeared after several minutes. It is important to point out that, according to Sklar *et al.* (1986), isoamylacetate is a poor stimulator of adenylate cyclase activity in ciliary membranes, and butanol, as other chemical solvents, does not stimulate. Furthermore, the weak stimulation of the adenylate cyclase activity

induced by isoamylacetate is observed at concentrations that are one order of magnitude higher than those used by Trotier (1986). Thus, it is conceivable that the response to these odorants reported by Trotier (1986) might involve a transduction mechanism that is not mediated by odorant activation of the adenylate cyclase activity and involves, in consequence, a conductance pathway different from the cyclic nucleotide-gated conductance described by Nakamura and Gold (1987).

Odorant responses have also been recorded in isolated olfactory neurons from mice by Maue and Dionne (1987). Unfortunately, because of technical difficulties, they could not use the whole-cell-recording technique and had to limit themselves to study the effects of odorants in cell-attached patches by monitoring single-channel activities, current noise, and holding current level. Exposing the cells to a mixture of odorants made of heptane, isobutyl alcohol, ethyl acetate, and benzaldehyde at concentrations of 10–100 $\mu$M produced the development of 5- to 50-pA inward currents, increments in the peak-to-peak noise of the holding current, and increases in single-channel activity. None of the odorants used in these studies have been tested for their ability to stimulate adenylate cyclase activity in ciliary membranes. However, none of a variety of organic solvents tested by Sklar *et al.* (1986) were effective in stimulating the adenylate cyclase in olfactory cilia. Therefore, it is difficult to establish whether the inward currents and the increase in channel activity measured by these authors in response to the odorants bear any relation to the cyclic nucleotide-gated conductance pathway described by Nakamura and Gold (1987).

The odorant-induced inward currents measured by Maue and Dionne (1987) developed 1 or 2 sec after the application of odorants and lasted 1–15 sec. Cells became unresponsive to odorants after a few consecutive exposures to the sensory stimuli. Forskolin, an activator of adenylate cyclase activity used in conjunction with Ro20-1724, an inhibitor of the phosphodiesterase activity, induced similar changes to those observed as a result of exposure to odorants in two of ten cells. At 100 $\mu$M concentration, odorants elicited responses in a large fraction (one-third) of cells. The results reported by Trotier (1986) and by Maue and Dionne (1987), although preliminary, are encouraging since they demonstrate that isolated vertebrate olfactory cells preserve their ability to respond to odorants.

### *Single-Channel Currents from the Dendrite and Soma of Olfactory Cells*

Records of single-channel currents from isolated olfactory receptor cells have shown the presence of several channels selective to potassium ions. Trotier (1986) recorded a $K^+$-selective channel in olfactory cells from the

salamander. In outside-out excised patches and under conditions in which the patch pipette was filled with a solution mimicking the composition of the cytosol and normal Ringer in the outer solution, the channel conductance is 34 pS and the channel is potassium selective. The channel displays open-time probabilities that are not appreciably voltage dependent, spending 70–80% in the open state and with open times of about 54 msec. In cell-attached patches, Trotier (1986) recorded channels with conductances of 20–38 pS that were blocked by TEA and $Cs^+$, and whose open-time probabilities increased at hyperpolarizing voltages.

A more detailed study on $K^+$-selective channels present in the soma and dendrite of isolated olfactory cells from mice has been published by Maue and Dionne (1987). Two of these $K^+$ channels are activated by calcium. They exhibit different affinities for calcium and different conductances. The largest one has a conductance of 130 pS (conductance estimated in symmetrical 140 mM $K^+$); it was recorded both in cell-attached and excised patches and is present in the soma and dendrite (Fig. 3A). The channel displays voltage-dependent kinetics, a linear current-voltage relation, and $P_K/P_{Na} = 7$, as estimated from the Goldmann–Hodgkin–Katz equation. This channel is blocked by intracellular $Cs^+$, but not by TEA or 4-amino-pyridine. Activation by calcium is observed at micromolar concentrations with opening probabilities increasing from 10% at 0.5 $\mu$M to 90% at 1 $\mu$M. At 0.7 $\mu$M calcium, the channel spends nearly 50% of the time in the open state. Calcium and membrane depolarization increase mean open times (fivefold every 150 mV). Identical channels were obtained in olfactory cells from neonatal mice.

The second type of $Ca^{2+}$-activated $K^+$-selective channel found in mice displays an intermediate open conductance of 80 pS (measured in symetrical solutions containing 140 mK $K^+$) (Fig. 3B). The channel has a peculiar flickery kinetic behavior and is present both in the somatic and dendritic membranes. The channel has linear I-V curve and a $P_K/P_{Na} = 10$. Calcium activation occurs at higher concentrations than those required to activate the large, 130-pS $Ca^{2+}$-activated $K^+$-selective channel, and is blocked by intracellular $Cs^+$.

A third type of $K^+$-selective channel found in mouse olfactory receptors is not affected by intracellular calcium, and displays voltage dependence and inactivation (Fig. 3C). The channel has a conductance of 41 pS under conditions in which the patch pipette contains 140 mM $K^+$ buffer and 39 pS when the pipette contains normal extracellular Ringer solution. No blocking agents were tested in this particular case. Depolarizations $> 30$–40 mV are required to activate this channel, with channel open times increasing and time latencies for the first opening decreasing at larger depolarizations. An

interesting feature of this channel is that it inactivates within 300–900 ms after a depolarization. The 40-pS potassium channel was recorded from olfactory receptors obtained from both adult and neonatal mice. In addition, a 20- to 25-pS $K^+$ channel exhibiting long-lasting open times was also recorded from the soma and dendrite of olfactory cells. Channel activity did not depend on intracellular calcium, but could not be maintained in excised patches. The channel exhibits a nonlinear I-V curve rectifying at depolarizing voltages both in cell-attached and excised patches. On the other hand, the channel is mostly closed at depolarizing voltages, and open-time probabilities increase with hyperpolarization. Open-time durations increase *e*-fold every 30 mV.

Mauer and Dionne (1987) have also recorded calcium and chloride channels in mouse isolated receptors. $Ca^{2+}$ channels seem to activate transiently at depolarizing voltages both in the soma and in the dendritic knob, and have a conductance of 60 pS under conditions in which the patch pipette contains 60 mM $BaCl_2$. Large 210-pS anion-selective channels were also detected both in the soma and the dendrite. The channels display a weak voltage dependence with open times increasing at depolarizing voltages. Intracellular calcium does not affect the behavior of this anion-selective channel.

## Reconstitution of Chemosensory Membranes in Planar Lipid Bilayers

### *Channel Reconstitution from a Crude Rat Olfactory Homogenate*

The reconstitution of membrane fragments derived from olfactory tissue in planar lipid bilayers was pioneered by Vodyanoy and Murphy (1983), who fused a crude homogenate obtained from rat olfactory mucosa to solvent-free planar bilayers. The rat olfactory homogenate (ROH) consists mostly of vesicles 30–50 $\mu$m in diameter as shown in negative-stain electron micrographs. Unfortunately, this preparation has not been biochemically characterized, and its content of chemosensory ciliary membranes cannot be assessed. In their original experiments, Vodyanoy and Murphy (1983) suspended the homogenate in a buffer containing 15 $\mu$M ATP and 10 $\mu$M GTP. Aliquots of this homogenate (3 $\mu$g protein) were added to one side of a planar bilayer.

The bilayer chamber contained 20 mM NaCl, 20 mM KCl, and 2 mM $CaCl_2$ at pH 7.4. The final protein, GTP, and ATP concentrations in these reconstitution experiments cannot be estimated since the authors did not report the volume of the bilayer chamber to which the ROH was added. Addition of the homogenate to the bilayer chamber resulted in the appearance of current fluctuations reflecting the insertion of ion channels to the

bilayer. The fluctuations decayed to nearly zero within 20 min. Addition of 25 nM diethyl sulfide to the same side of the bilayer to which the ROH had been added resulted in activation of a new set of current fluctuations. Both in the absence and in the presence of odorant, the channel displayed slow kinetics. Mean open times were estimated at 29 sec in the absence of diethyl sulfide and at 42 sec in its presence. Open-state probabilities of 0.66 and 0.80 were measured in the absence and in the presence of odorant, respectively. The channel was reported to be selective to potassium ions with a conductance of 60 pS. Activation of a similar channel was also demonstrated in the presence of the odorant (−)carvone.

More recently, Vodyanoy and Vodyanoy (1987) have reported that the steady-state conductance of planar lipid bilayers exposed to ROH becomes sensitive to diethyl sulfide only in the presence of ATP and GTP. Furthermore, according to these authors, odorant effect on bilayer conductance could be mimicked cAMP. Peculiarly, ATP, GTP, cAMP, and diethyl sulfide exert their effects when added to the same bilayer side to which the vesicles are added. Thus, the site of action of cAMP and odorant presumably corresponds to the cytoplasmic side of the conductance pathway. The authors did not clarify in their report whether the activation of bilayer conductance by odorants and cAMP is reversed upon perfusing the bilayer chamber with cAMP-free solution, making it diffult to assess whether cAMP acts directly or via a cAMP-dependent kinase.

### *Channel Reconstitution from Partially Purified Olfactory Ciliary Membranes*

The development of procedures to obtain a highly enriched membrane preparation of olfactory cilia from frog olfactory epithelium paved the way to reconstitution studies in a preparation that had already been subjected to careful biochemical scrutiny (Anholt *et al.*, 1986, 1987; Sklar *et al.*, 1986). Cilia detached from amphibian olfactory epithelium (Anholt *et al.*, 1986; Pace *et al.*, 1985) undergo osmotic lysis, forming isolated axonemal structures and membrane visicles with diameters of 100–500 nm with a yield of $\sim 200\ \mu g$ protein/frog. The most relevant information obtained from biochemical studies in isolated olfactory cilia membranes is the discovery that it contains a high activity of adenylate cyclase that can be stimulated to different extents by many, but not all, odorants (Sklar *et al.*, 1986). Activation requires the presence of GTP in the assay (Pace *et al.*, 1985; Sklar *et al.*, 1986). Adenylate cyclase activity in isolated cilia is 150 times higher than in the olfactory epithelium, indicating that the preparation is highly enriched in chemotransductory membranes, making it an ideal material for reconstitution studies in planar lipid bilayers.

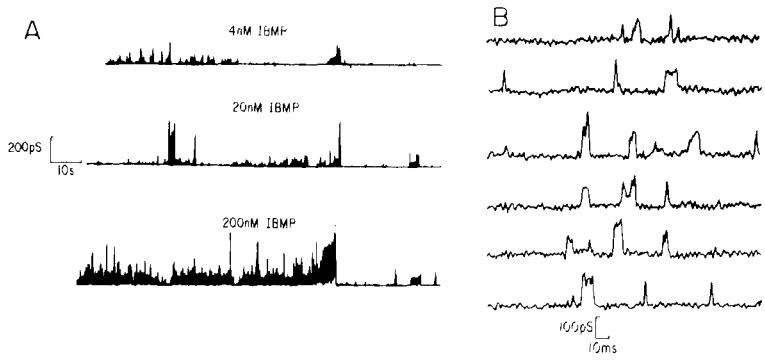


**Fig. 4.** Activation of odorants of cationic channels from olfactory cilia in planar lipid bilayers. (A) Activation of channels by the bell pepper odorant 3-isobutyl-2-methoxypyrazyne (IBMP) is proportional to odorant concentration. Both compartments in the experimental chamber contained 0.2 M sodium acetate, 5 mM HEPES, and 0.5 mM EGTA at a pH of 7.0. Voltage difference: 50 mV. All records were obtained from the same membrane. (B) Channels under similar conditions (30 nM IBMP) are shown at higher temporal resolution. Bandwidth: 500 Hz. Single-channel conductance: 90 pS. Modified from Labarca *et al.* (1988).

Labarca *et al.* (1988) fused ciliary membranes obtained from the olfactory epithelium of *Rana catesbeiana* to Mueller–Rudin-type planar lipid bilayers (Mueller and Rudin, 1969) made of a mixture of phosphatidylethanolamine and phosphatidylserine. Fusion required the presence of calcium and osmotic gradients, and resulted in the insertion of several conductance pathways. The most relevant observation contributed by these studies was the detection of a cation-selective multiconductance channel that could be activated directly and reversibly by nanomolar concentrations of the bell pepper odorant IBMP (3-isobutyl,2-methoxipyrazine) and the citrus odorant citralva (3,7-dimethyl-2,6-octadienenitrile) (Fig. 4A). The channel was found not to discriminate between sodium and potassium ions, based on measurements of permeability ratios. Activation was observed in the absence of cyclic nucleotides. Channel activity induced by galbazine and citralva consisted of discrete bursts that tended to decay within minutes. Conductance histograms of discrete bursts revealed the presence of 35-pS substates (measured in 200 mM NaCl). The highest conductance achieved within a burst had a value of 420 pS. Activation by odorants was dose dependent in such a way that the probability of reaching the higher conductance levels increased with odorant concentration. Galbazine was found to be a more potent activator of channel activity than was citralva. Thus, while galbazine was able to activate bursts of activity at concentrations of $< 4$ nM, activation by citralva required concentrations of $\sim 1$ order of magnitude higher.

Several minutes after odorant addition, bursts of activity were no longer observed. Bursts often terminated abruptly and were followed by a diminished single-channel-like activity with average conductances of 90 pS and lifetimes in the millisecond range (Fig. 4B). Control studies showed that fusion of membrane fragments derived from frog respiratory cilia did not incorporate odorant-activated channels to the planar bilayer. The report by Labarca *et al.* (1988) did not include studies on the effects of cyclic nucleotides or other putative second messengers on the odorant-activated channels, or studies on the activation of this or different conductance pathways by other odorants or second messengers.

In addition to the odorant-activated channel, three other conductance pathways were inserted upon fusing olfactory cilia membranes to planar bilayers. The most frequently recorded one corresponds to a $K^+$-selective channel having a conductance of 190 pS (in 200 mM KCl). The channel's selectivity sequence, derived from permeability ratio estimates under biionic conditions, was found to be $K^+ > Rb^+ > NH4^+ \gg Na^+, Cs^+$. The large $K^+$-selective channel usually coinserted in the planar bilayer with the odorant-gated one. Thus, its occurrence was taken as evidence for the incorporation of odorant-activated conductances.

A second, commonly inserted conductance was a large, anion-selective channel that has not been further characterized. To avoid it during the studies of cation-selective channels, experiments were usually carried out in the presence of acetate salts. However, it should be pointed out that Maue and Dionne (1987) have reported the presence of large, anion-selective channels in the soma and dendrite of mouse olfactory neurons. A fourth channel that occurred less frequently in planar bilayer experiments was cation selective with a conductance of 40 pS (in 0.2 M KCl). The channel exhibits burst kinetics and a low probability of opening, and does not seem to select between sodium and potassium ions. Neither the $K^+$-selective channel nor the cation-selective 40-pS channel were affected by the two odorants used.

An interesting outcome from the bilayer experiments is the suggestion that cilia from olfactory receptors contain channels other than those directly involved in transduction, a finding that is consistent with the results reported by Nakamura and Gold (1987). Ciliary membrane might contain few non-trasductory channels, as compared with a large density of channels involved in transduction.

The information already available on macroscopic and single-channel currents can be integrated to hypothesize on the role they play in determining the electrical response of olfactory receptor neurons to odorants. Events will start by the opening of excitatory channels triggered either by an increase in second-messenger concentration or directly by odorants, giving rise to the

receptor potential. The resulting depolarization will lead to the opening of calcium channels in the dendrite and soma, initiating an action potential. Calcium channel inactivation and opening of $K^+$ channels will transiently repolarize the membrane. The $K^+$-selective channel that inactivates within the first few hundred milliseconds will be important early in the response of the olfactory neuron by contributing to its repolarization after the first action potentials, which are of larger amplitude. The persistence of a steady excitatory current in the cilia will maintain the membrane potential above threshold and lead to repetitive firing in the dendrite and soma. The decrement in spite amplitude observed during a prolonged period of exposure to odorant might be exlained by partial inactivation of calcium channels in the dendrite and soma. On the other hand, opening of $Ca^{2+}$-activated $K^+$ channels, due to an increase in intracellular calcium, will determine the plateau of the receptor potential.

## Conclusions and Future Prospects

The availability of simple procedures to isolate functional vertebrate olfactory receptor neurons from several species has rendered them amenable to patch-clamp recording. The patch-clamp technique has already been used to record currents, both at the macroscopic and single-channel levels from the dendrite and soma of isolated olfactory cells obtained from salamanders, frogs, and mice and from the cilia of frog receptors. Whole-cell recording allows for true voltage clamping of isolated olfactory cells and renders the intracellular milieu accessible through the patch pipette, making it possible to dialyze the cell with second messengers, calcium, and other agents of interest. As shown by Trotier (1986), odorant responses can be monitored under voltage clamp, and the ionic basis of receptor excitability can be scrutinized, althouth conditions for long and steady recording are not yet satisfactory. On the other hand, use of the cell-attached and excised patch modalities enable the recording of single-channel currents under well-defined experimental conditions. A critical question that remains to be answered concerning the primary steps involved in odor transduction is the mechanism by which odorant binding activates channels in the ciliary membrane. The study by Nakamura and Gold (1987) demonstrated that the cilia, dendrite, and soma of frog olfactory cells possess a cyclic nucleotide-gated, cation-selective conductance. Pharmacological studies aimed at identifying blockers of the cyclic nucleotide-gated conductance will be crucial in evaluating its role in olfactory transduction. The availability of blockers of the cyclic nucleotide-gated conductance would enable electrophysiological studies

directed at distinguishing between odorants whose transduction mechanisms involve a putative common second messenger from those that operate through alternative mechanisms. Inside-out patches excised from the cilia, and perhaps from the dendritic knob, should enable direct testing of the idea suggested by reconstitution studies—that some odorants might directly gate ion channels in the ciliary membrane.

An alternative experimental approach for studying conductance mechanisms in olfactory cilia membranes and their regulation by odorants, second messengers, and ions is the reconstitution of vesicular membrane fragments derived from isolated cilia in planar lipid bilayers. This approach has proved to be a simple and powerful tool to examine ionic conductances in biological membranes in which other electrophysiological techniques are difficult or impossible to use (Coronado and Labarca, 1984; Miller, 1986). Reconstitution of ion channels in planar lipid bilayers has many advantages. It is a simple and reproducible assay. Both sides of the bilayer are easily accessible and can be perfused several times, making it possible to investigate a single-channel molecule under different conditions during the same experiment. Reconstitution studies are expected to provide a characterization of ion channels present in olfactory cilia, particularly now that a highly purified preparation of olfactory cilia membranes is available. Planar bilayer reconstitution is particularly suited to investigate the possible modulation of channel activity by odorants and second messengers. Future work in planar bilayers should concentrate in further studying the properties of the already identified odorant-gated multiconductance cation channels in order to define which of a variety of odorants are effective in activating this conductance pathway and whether or not cAMP and other second messengers are effective in modulating channel activation. Pharmacoligical characterization of this channel will be crucial in identifying specific blockers because it would help relate the bilayer work with electrophysical studies in the olfactory epithelium or in isolated olfactory cells. Obviously, blockers of odorant-gated channels in planar bilayers would predictably abolish the response of the olfactory epithelium to those odorants known to gate them. Identifying high-affinity blockers of the odorant-gated conductance would also be important to those interested in attempting the purification and reconstitution of these molecular entities. In addition, bilayer reconstitution studies should also help in identifying additional conductance pathways activated by odorants as well as in investigating the properties of ion channels present in olfactory cilia which, even if not directly involved in transduction, would contribute to an understanding of the electrical properties of the ciliary membrane. In summary, one would predict that, in the next few years and as a result of combining patch-clamp

and reconstitution approaches, our knowledge of the mechanisms that operate in the primary events of olfactory chemotransduction, as well as the regulation of the electrical activity of olfactory receptor neurons, will be greatly expanded.

## Acknowledgments

We thank Drs. Javier Alvarez-Leefmans, Osvaldo Alvarez, and Ramón Latorre for useful suggestions. This work was supported by grants from Tinker Foundation, NIH (GM-35981), and Fondo Nacional de Ciencia y Tecnología 88/1167 and Grant SS11107 from the O.N.R.

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