

# Transduction Mechanisms in Vertebrate Olfactory Receptor Cells

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**Schild, Detlev, and Diego Restrepo.** Transduction Mechanisms in Vertebrate Olfactory Receptor Cells. *Physiol. Rev.* 78: 429–466, 1998.—Considerable progress has been made in the understanding of transduction mechanisms in olfactory receptor neurons (ORNs) over the last decade. Odorants pass through a mucus interface before binding to odorant receptors (ORs). The molecular structure of many ORs is now known. They belong to the large class of G protein-coupled receptors with seven transmembrane domains. Binding of an odorant to an OR triggers the activation of second messenger cascades. One second messenger pathway in particular has been extensively studied; the receptor activates, via the G protein  $G_{olf}$ , an adenylyl cyclase, resulting in an increase in adenosine 3',5'-cyclic monophosphate (cAMP), which elicits opening of cation channels directly gated by cAMP. Under physiological conditions,  $Ca^{2+}$  has the highest permeability through this channel, and the increase in intracellular  $Ca^{2+}$  concentration activates a  $Cl^-$  current which, owing to an elevated reversal potential for  $Cl^-$ , depolarizes the olfactory neuron.

The receptor potential finally leads to the generation of action potentials conveying the chemosensory information to the olfactory bulb. Although much less studied, other transduction pathways appear to exist, some of which seem to involve the odorant-induced formation of inositol polyphosphates as well as  $\text{Ca}^{2+}$  and/or inositol polyphosphate-activated cation channels. In addition, there is evidence for odorant-modulated  $\text{K}^+$  and  $\text{Cl}^-$  conductances. Finally, in some species, ORNs can be inhibited by certain odorants. This paper presents a comprehensive review of the biophysical and electrophysiological evidence regarding the transduction processes as well as subsequent signal processing and spike generation in ORNs.

## I. INTRODUCTION

The morphology of olfactory receptor neurons (ORNs) was first described by Schultze (314, 315; reviewed in Ref. 366) 140 years ago. How ORNs function, however, has remained a riddle that still is not completely solved. In 1985 and 1986, research on olfactory transduction took a decisive turn. Two reviews published by Getchell and Lancet described the functional properties of vertebrate ORNs (106) and the processes in olfactory perception ranging from biochemistry to systems theory (188). This wealth of knowledge had been accumulated using classical morphological and biochemical methods and classical electrophysiological tools, above all extracellular recordings. Anderson and Ache's study of 1985 (in the lobster) (7) and Trotier's study of 1986 (in the salamander) (343) were the first to report the use of the patch-clamp technique to provide a description of voltage-gated membrane currents, single-channel recordings, and odorant-induced currents in ORNs. The various patch-clamp recording configurations (115), other biophysical methods, and molecular biological techniques allowed a more detailed study of signal transduction in the ORN. Since then, a number of reviews and minireviews have appeared, each dealing with a specific topic in olfactory perception, e.g., perireceptor events (107, 270, 271), the structure and function of receptor proteins (52, 189, 283), ultrastructural studies (232, 233), second messenger signaling (2, 9, 10, 40, 43, 47, 74, 295, 298, 372), cyclic nucleotide-gated channels (151, 357, 369, 372),  $\text{Ca}^{2+}$ -dependent  $\text{Cl}^-$  channels in ORNs (185), and adaptation and concentration coding (340, 344) as well as aspects of coding and information conveyance between olfactory epithelium and olfactory bulb (52, 149, 189, 247, 252, 304, 316, 322). However, a comprehensive review of cellular studies of olfactory transduction focused on electrophysiological and biophysical aspects is lacking.

Here we review the biophysical, electrophysiological, and some of the biochemical work done in ORNs over the past 10 years. Although we focus on the functional aspects of vertebrate olfactory transduction, we point out interesting parallels to chemosensory transduction mechanisms in invertebrate ORNs (2, 3, 137, 139, 140, 329), eukaryotic microorganisms (346), and vomeronasal receptor neurons (197). Molecular biological studies are mentioned where appropriate but are not covered comprehensively.

The reader is referred to recent reviews covering biochemical and molecular biological aspects of olfactory transduction (2, 47, 52, 189, 247, 295, 298, 322). This review of electrophysiological and biophysical studies documents significant advances in our understanding of olfactory transduction in the last 10 years. More importantly, the comprehensive review of the literature makes a compelling argument that olfactory receptors are more complicated than heretofore imagined and reveals the limitations in our knowledge of the complexity of the mechanisms underlying olfactory transduction.

## II. GEOMETRY AND PASSIVE MEMBRANE PROPERTIES OF OLFACTORY RECEPTOR NEURONS

### A. Geometry

Olfactory receptor cells in vertebrates are bipolar neurons with a small soma, a single dendrite, cilia or microvilli attached to the olfactory knob or vesicle formed at the dendritic end, and an axon projecting to the olfactory bulb (Fig. 1). The soma has a round or ellipsoidal shape with the short and long axes ranging from 4 to 15  $\mu\text{m}$  and from 7 to 21  $\mu\text{m}$ , respectively. Among the species studied using electrophysiological and biophysical techniques, the largest ORNs are found in newts (182) and salamanders [*Ambystoma tigrinum* (92) and *Necturus maculosus* (76)], whereas the smallest ORNs are found in zebrafish (*Danio rerio*) (65). The unbranched axon is  $\sim 200$  nm in diameter, and its length, when assessed in isolated cell preparations, varies considerably. The dendrite is  $\sim 1\text{--}3$   $\mu\text{m}$  thick and between 5 and 120  $\mu\text{m}$  long (253, 284). In a preparation of isolated receptor cells, the dendrites may shorten either due to the preparation procedure (307) or due to an excessive  $\text{Ca}^{2+}$  load (310). The dendrite ends in a dendritic knob (diameter 2–3  $\mu\text{m}$ ) from which several cilia issue. The number of cilia appears to vary approximately between 5 and 40 (76, 147, 148, 236, 253, 284). The diameter of a cilium is 100–250 nm (depending on the species), i.e., at or below the resolution limit of light microscopes, and its length is in the range between 5 and 250  $\mu\text{m}$  (124, 166, 279, 284, 371). A cilium is thicker at its base and tapers down its length somewhat (231–233, 318). The typical ciliary 9 + 2 structure changes over the length of a cilium, because the

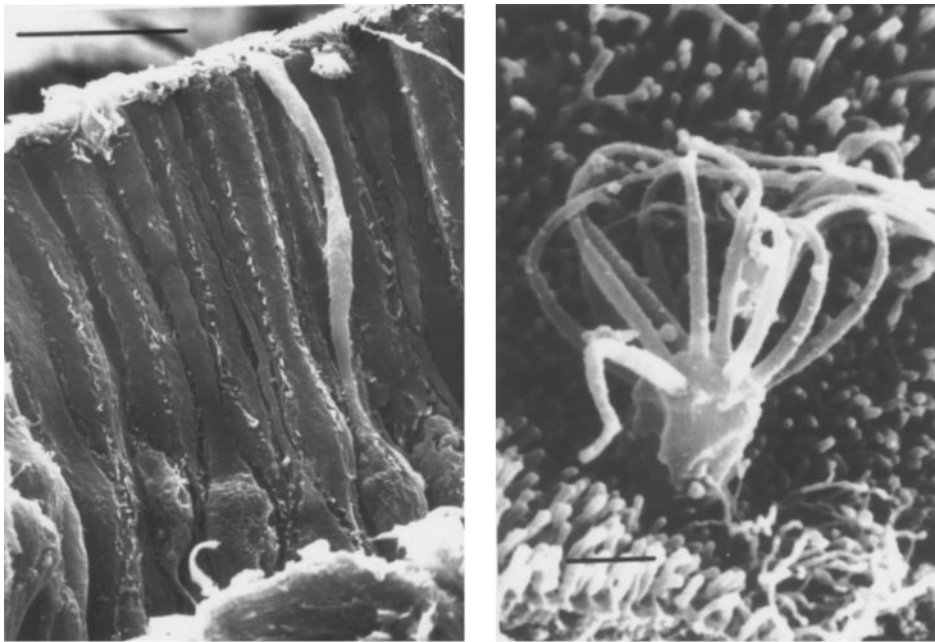
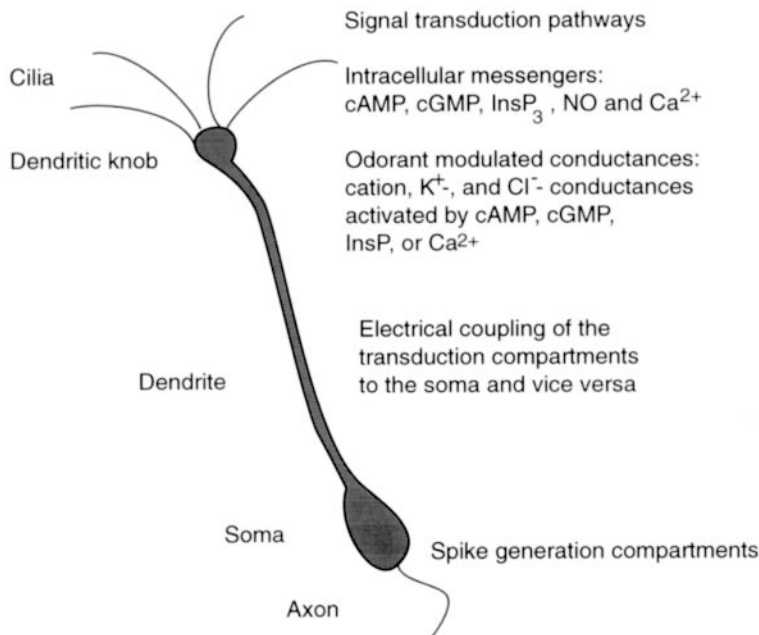


FIG. 1. *Top*: scanning electron micrographs of human olfactory receptor cells. *Left*: olfactory mucosa viewed from side. Cell with long, slightly prominent dendrite is a typical olfactory neuron. Scale bar, 10  $\mu\text{m}$ . *Right*: view of surface of olfactory mucosa shows an olfactory knob with 8 cilia. Scale bar, 1  $\mu\text{m}$ . [From Morrison and Costanzo (253).] *Bottom*: schematic diagram of an olfactory neuron showing transduction compartments (cilia and dendritic knob), dendrite, soma, and beginning of axon as well as some of the characteristic properties of compartments.  $\text{InsP}_3$ , inositol trisphosphate; NO, nitric oxide.



number of microtubules is reduced. In frog, short and medium-sized (up to 90  $\mu\text{m}$ ) cilia move irregularly, whereas the long cilia (up to 250  $\mu\text{m}$ ) do not move (124, 166). In mammals, the cilia are immotile (231).

A comparison of morphological data (124, 284) with electrophysiological reports clearly indicates a tendency for isolated cells to exhibit shorter cilia, although cilia as long as 130  $\mu\text{m}$  have been found and recorded from (166). Occasionally, isolated ORNs lose all the cilia during the dissociation procedure. Deciliated olfactory receptor cells of the newt do not respond to odors (182). For a survey

of the electrical properties of olfactory cilia in frog, see References 159, 164, 166.

Often during recordings in the whole cell mode of the patch-clamp technique, the seal with the patch pipette is established on the soma, which is the part of the cell most distant from the cilia. Because of this, space-clamp effects due to the fact that the resistance of the plasma membrane is finite can come into play, particularly in large ORNs with long cilia during odorant stimulation. The effects of space clamp in ORNs have been described by various investigators (166, 213, 276).

The receptor cells in vomeronasal organs (6, 20, 171, 318, 319) and a number of receptor cells in the olfactory epithelium of fish (78, 128, 250, 254, 338, 358, 367, 368), reptiles (112, 352), and amphibia (171) bear microvilli instead of cilia. Microvillous cells have also been reported in the olfactory epithelium of mammals, in particular, in humans (242, 251, 253, 300). There are no reports on their function, and there is controversy on whether they project axons to the olfactory bulb. Thus one study found that when horseradish peroxidase (HRP) is injected into the olfactory bulb, HRP reaction products can be found with a certain delay in microvillous cells of the olfactory epithelium (300), whereas another study failed to find axonal processes stemming from putative microvillar cells in rat (54). In the vomeronasal organ of mouse and rat, two different subclasses of receptor neurons have been described that express two different G proteins and project differentially to the accessory olfactory bulb (131). In the goldfish, there is a correlation between the reappearance of microvillous cells after axotomy and the behavioral responses to pheromones (368). Interestingly, ORNs and axons from the different kinds of chemosensory neuroepithelia, i.e., olfactory proper versus accessory olfactory or vomeronasal mucosa, can be labeled differentially by lectin agglutinins (94, 95, 239).

## B. Capacitance and Resting Membrane Resistance

The resting electrical properties including the capacitance ( $C$ ), the resting membrane resistance ( $R_m$ ), and the resting potential ( $u_r$ ) of ORNs have been measured in various vertebrate and invertebrate species. The measured capacitance of ORNs (Table 1) is in the range from 0.7 to 35 pF. The smallest and the largest values have been found in zebrafish (65) and in newt (F. Kawai, personal communication), respectively. With the assumption of a standard value of  $1 \mu\text{F}/\text{cm}^2$  (corresponding to  $1 \text{ pF}/100 \mu\text{m}^2$ ), the membrane surface of ORNs is thus in the range between 70 and  $3,500 \mu\text{m}^2$ .

In patch-clamp recordings from ORNs, the seal resistance and the resting membrane resistance are often in the same range (between 1 and  $40 \text{ G}\Omega$ ). This can lead to problems in the interpretation of data, and it is therefore particularly important to make a clear distinction between the resting input resistance ( $R_i$ ) and  $R_m$ . The  $R_i$  value is usually measured in the whole cell configuration using voltage clamp. Starting at a holding voltage of about  $-80 \text{ mV}$ , a small voltage pulse  $\Delta u$  of typically  $-10 \text{ mV}$  is applied, and the resulting asymptotic current shift  $\Delta I$  is measured. The  $\Delta u/\Delta I$  determined at  $u_r$  gives the  $R_i$ , which is the combined resistance of  $R_m$  and the seal resistance ( $R_{\text{seal}}$ )

$$R_i = (R_m R_{\text{seal}})/(R_m + R_{\text{seal}}) \quad (1)$$

TABLE 1. *Input resistance and capacitance values for olfactory receptor neurons*

Species	Whole Cell Capacitance, pF	Reference No.
Zebrafish ( <i>Danio rerio</i> )	0.7	65
Catfish ( <i>Ictalurus punctatus</i> )	2	246
Rat ( <i>Rattus rattus</i> )	2–4	219, 264
Human ( <i>Homo sapiens</i> )	4	292
Squid ( <i>Loligo opalescens</i> )		
Floriform cells	5.7	217
Piriform cells	11.9	
Clawed frog ( <i>Xenopus laevis</i> )	4–7	305
Chilean toad ( <i>Caudiverbera caudiverbera</i> )	5	69, 249
Salamander ( <i>Salamandra salamandra</i> )	6–11	343
Mudpuppy ( <i>Necturus maculosus</i> )	16	76
Salamander ( <i>Ambystoma tigrinum</i> )	21	92
Newt ( <i>Cynops pyrrhogaster</i> )	35	F. Kawai, personal communication

whereby the series (or access) resistance ( $R_s$ ) of the pipette is neglected here because it should be at least two orders of magnitude smaller than  $R_m$  or  $R_{\text{seal}}$ . From Equation 1 it follows that the  $R_m$  can be obtained from  $R_i$  and  $R_{\text{seal}}$  (as measured in the cell-attached mode)

$$R_m = R_i/(1 - R_i/R_{\text{seal}}) \quad (2)$$

Seal resistances as reported in recordings from ORNs are in the range between  $1 \text{ G}\Omega$  (182) and  $53 \text{ G}\Omega$  (219). Clearly, the approximation  $R_m \sim R_i$  is better the smaller the  $R_i/R_{\text{seal}}$ .

## C. Resting Membrane Potential

The resting membrane potential  $u_r$  in ORNs, measured as the zero-current potential in the whole cell configuration of the patch-clamp technique, has been reported in the remarkably large range between  $-90 \text{ mV}$  (98) and  $-30 \text{ mV}$  (305). These widely differing values might be explained by species differences. However, Firestein and Werblin (92) explained the deviation of  $u_r$  (in their study  $-54 \text{ mV}$ ) from the  $\text{K}^+$  equilibrium potential ( $E_K$ ; in their study  $-98 \text{ mV}$ ) by the membrane being permeable for ions other than  $\text{K}^+$ . A deviation of  $u_r$  from  $\text{K}^+$  potential ( $u_K$ ) could be brought about by three other conductances.

First, a  $\text{Cl}^-$  conductance (76, 165) with  $\text{Cl}^-$  potential ( $u_{\text{Cl}} > E_K$ ) as reported in ORNs of the mudpuppy (76), newt (184), and the clawed frog (361) would raise  $u_r$ . In the frog, this explanation might, however, be incorrect because the  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  conductance ( $g_{\text{Cl}}$ ) is half-

activated at intracellular  $\text{Ca}^{2+}$  concentrations of  $\sim 5 \mu\text{M}$  (165), which is almost two orders of magnitude higher than the resting  $\text{Ca}^{2+}$  concentrations in ORNs as obtained in imaging studies in frog (309–311). Hence, the  $\text{Ca}^{2+}$ -dependent  $\text{Cl}^-$  conductance in frog is presumably not activated at rest.

A second conductance that necessarily affects the measurements of  $u_r$  is the seal between plasma membrane and patch pipette. With  $g_{\text{seal}} (=1/R_{\text{seal}})$  and  $g_{\text{Ko}} (=1/R_{\text{Ko}})$  being the seal conductance and the resting  $\text{K}^+$  conductance, and with the assumption that other conductances are negligible at rest, the resting membrane potential is given by the equation (113)

$$u_r = f_{\text{seal}}u_{\text{seal}} + f_{\text{Ko}}u_{\text{K}}$$

where  $f_{\text{seal}} = g_{\text{seal}}/(g_{\text{seal}} + g_{\text{Ko}})$ ,  $f_{\text{Ko}} = g_{\text{Ko}}/(g_{\text{seal}} + g_{\text{Ko}})$  are the fractional conductances with  $u_{\text{seal}}$  and  $u_{\text{K}}$  being the corresponding equilibrium potentials. Because  $u_{\text{seal}} \sim 0 \text{ mV}$ ,  $u_r$  can be approximated by

$$u_r = f_{\text{K}}u_{\text{K}} = u_{\text{K}}g_{\text{Ko}}/(g_{\text{seal}} + g_{\text{Ko}}) = u_{\text{K}}/(1 + R_{\text{Ko}}/R_{\text{seal}}) \quad (3)$$

This shows that a “good seal resistance” is a relative concept and that  $u_r$  is reliably determined only if  $R_{\text{seal}} \gg R_{\text{Ko}}$ , a requirement difficult to meet in small neurons with a high resting resistance.

Frings and Lindemann (99), for example, have reported  $u_r$  approximately equal to  $-85 \text{ mV}$  with  $R_i \sim 5 \text{ G}\Omega$  and  $R_{\text{seal}} \sim 40 \text{ G}\Omega$ . Indeed, with  $u_{\text{K}} = -91 \text{ mV}$ , Equations 2 and 3 give  $R_{\text{Ko}} \sim 7.5 \text{ G}\Omega$  and  $u_r = -79 \text{ mV}$ , which is in the range of the measured value. Whenever  $R_i$  and  $R_{\text{seal}}$  happen to be of the same order of magnitude, the seal short circuits the membrane potential. For example, with  $R_i = 4 \text{ G}\Omega$ ,  $R_{\text{seal}} = 10 \text{ G}\Omega$ , and  $u_{\text{K}} = -97 \text{ mV}$ , Equations 2 and 3 yield  $u_r = -57 \text{ mV}$  (90). The short-circuiting effect of the seal shunt has been modeled and analyzed in detail by Pongracz et al. (276).

An additional problem arises if the seal dependence of a measured resting membrane potential affects further steps of data evaluation. For example, to investigate the  $\text{Cl}^-$  equilibrium potential in mudpuppy ORNs, Dubin and Dionne (76) recorded the  $\text{Cl}^-$  channel blocker-dependent shift  $\Delta u$  of current-voltage ( $I$ - $V$ ) curves recorded in the cell-attached mode. The resulting value  $\Delta u$  added to the resting membrane potential as subsequently measured in the whole cell mode (in this case  $u_r$  was  $-40 \text{ mV}$ ) gave  $u_{\text{Cl}} = -45 \text{ mV}$ . However, the seal-dependent inaccuracy in the measurement of  $u_r$  affects the resulting value for  $u_{\text{Cl}}$  so that  $u_{\text{Cl}}$  is more negative than  $-45 \text{ mV}$ , the exact value depending on  $R_{\text{seal}}$ .

A third conductance that can influence the measurement of  $R_m$  and  $u_r$  is an inward rectifying cation conductance ( $g_h$ ), which is permeable for  $\text{K}^+$  and  $\text{Na}^+$  ( $P_{\text{K}}/P_{\text{Na}} = 5$ ) and activates at hyperpolarized potentials. This conduc-

tance has been reported in ORNs of catfish (246), frog (343, 344), mouse (227), and rat (222). In other systems, a similar conductance is crucial for the generation of membrane potential oscillations (230). Whether or not this conductance underlies the membrane potential oscillations observed in ORNs (98, 205) is not known. Interestingly, however, Tokimasa and Akasu (339) have shown in sympathetic neurons that a conductance of the same type can be activated by increased levels of cAMP.

#### D. Membrane Time Constant

The product of the resistance  $R_m$  and the cell capacitance  $C$  determines the membrane time constant ( $\tau_m$ ). Average values of  $R_m \times C$  are  $\sim 60 \text{ ms}$ . The membrane can be regarded as an RC element or first-order low-pass filter with corner frequency ( $f_c$ ) of  $\sim 16 \text{ Hz}$  that dampens odor-induced current components for frequencies  $f > f_c$ . It thus rejects “high”-frequency noise (214). The time constants of ORNs were measured in various species using current injections into ORNs through a patch pipette. The values obtained with gigaohm seals are at least one order of magnitude larger than those obtained with sharp microelectrodes [e.g.,  $4.2 \text{ ms}$  in the tiger salamander (226)]. Owing to the high resting resistance and, at least in some species, a relatively high capacitance, the time constant  $\tau_m$  can be as long as  $\sim 100 \text{ ms}$  [e.g., in squid (217), or salamander (92)]. This implies that, because of the high  $R_m$ , ORNs can be excited by very small currents but that the depolarization is relatively slow because of the long time constants. This was nicely demonstrated by Lynch and Barry (219) as well as by Maue and Dionne (227), who observed that the current supplied by the opening of a single ion channel was able to trigger an action potential. The underlying membrane biophysics have been analyzed in detail by Lynch and Barry (219). The delay of the action potential after channel opening depended on the current amplitude. For the smallest current that elicited an action potential, it was in the range of the membrane time constant.

#### E. Sensitivity and Single Odorant Molecule Detection

The above-mentioned evidence that the current through a single channel can excite an olfactory neuron poses the question of whether a single odorant molecule binding to an odorant receptor can induce an action potential. Menini et al. (238) suggested this. These authors applied an odorant at concentrations well below the  $K_{1/2}$  for the dependence of odor-induced steady-state current on odor concentration. The resulting current fluctuations were stereotyped, “quantal-like” in amplitude ranging from  $0 \text{ pA}$  (failure) to  $9.5 \text{ pA}$ , with an average response

of  $\sim 1$  pA (holding potential,  $-50$  mV). Based on these data, Menini et al. (238) concluded that odors induce quantal-like current fluctuations presumably triggered by the binding of a single odorant molecule to its receptor. These data do, however, not prove that binding of one odorant molecule induces a quantal current. To show this, it would be necessary to demonstrate that the frequency of occurrence of the current fluctuations is equal to the frequency of binding of odor molecules to the receptor (23, 317). Moreover, the detection of a single molecule by a receptor cell would require that a quantal current could induce an action potential. Although this is not excluded, particularly in the case when amplification of channel activity occurs through opening of  $\text{Ca}^{2+}$ -activated conductances (see sects. v and vi), the effect of quantal currents on membrane potential was not analyzed by Menini et al. (238).

On the other hand, Lowe and Gold (215) have shown that low concentrations of odorants elicit random current fluctuations in olfactory receptor cells from rat. These authors argue convincingly that these current fluctuations are not because of single molecular events. They show that the power spectrum for odor-induced currents is identical to the power spectrum of the current induced by increases in the concentration of the second messenger cAMP in the absence of odorants. They conclude that the current fluctuations induced by odorants are due to noise intrinsic to the transduction mechanism (intrinsic noise) and suggest that quantal detection is not a common property of olfactory receptor cells. Under basal conditions, the intrinsic noise may not be apparent because the steep dependence of current on cAMP concentration (Hill coefficient of 2–4) acts like a threshold. Addition of odorant would then elevate the level of activity above the threshold, thereby unmasking the intrinsic noise signals.

However, because of the differences in the experimental designs of the experiments carried out by Menini et al. (238) on the one hand, and Lowe and Gold (215) on the other, the data provided by Lowe and Gold do not rule out the possibility that the current fluctuations in the Menini study may be due to single molecular events (see also Ref. 295).

## F. Summary

Olfactory receptor cells in vertebrates are bipolar neurons with a small ( $\sim 6 \mu\text{m} \times 13 \mu\text{m}$ ) soma, a single dendrite, cilia, or microvilli attached to the dendritic ending, and an axon projecting to the olfactory bulb. The dendrite is  $\sim 1$ – $3 \mu\text{m}$  thick and between 5 and 120  $\mu\text{m}$  long. The number of cilia appears to vary approximately between 5 and 40. The receptor cells in vomeronasal organs and a number of receptor cells in the olfactory epithelium of fish, reptiles, and amphibians bear microvilli

instead of cilia. The capacitance and resting resistance of ORNs appears to be in the range from 0.7 to 35 pF and from 1 to 40  $\text{G}\Omega$ , respectively. When one considers the artifacts (above all the seal resistance) that affect the measurement of the resting potential  $u_r$ ,  $u_r$  seems to be between  $-85$  and  $-70$  mV. Membrane time constants range from  $\sim 40$  to  $>100$  ms. These properties make ORNs extremely sensitive. Binding of a few or maybe only one molecule can excite an ORN.

## III. CONDUCTANCES INVOLVED IN ACTION POTENTIALS

Vertebrate olfactory receptor cells are neurons. They have an axon along which they send encoded information to the central nervous system (CNS). In this section we review some biophysical properties of the conductances that are involved in the generation of action potentials in ORNs.

As outlined in section II, the resting membrane potential appears to be determined by a very low resting  $\text{K}^+$  conductance, which consists of more than one type of channel: 1) an inward rectifying conductance  $g_h$  (222, 227, 246, 343) and 2) a slowly inactivating delayed rectifier type of  $\text{K}^+$  conductance that has been observed in squid (217), fish (65, 246, 260), Chilean toad (69), frog (305, 343), salamander (92), rat (220, 342), and human (292). At the single-channel level, a slowly activating  $\text{K}^+$  current has been described (220) that could underlie the resting delayed rectifier conductance.

### A. Voltage-Gated Sodium Currents

Excitation of an olfactory neuron generates a receptor potential, and when the membrane potential reaches the firing threshold,  $\text{Na}^+$  channels activate and initiate spike generation.

The reports of the voltage-gated  $\text{Na}^+$  conductances ( $g_{\text{Na}}$ ) in ORNs for various species differ in some respects. 1) In the tiger salamander (92) and rat (280), it has been reported to be relatively insensitive to tetrodotoxin (TTX), whereas it is not in other species (65, 69, 246, 305). 2) It activates at about  $-60$  mV in *Xenopus laevis* (305) and frog (277),  $-50$  mV in catfish (246) and Chilean toad (69),  $-30$  mV in squid (217) and salamander (92), and about  $-45$  mV in rat (280, 342), human (292), salmon (260), zebrafish (65), and mudpuppy (76). This variability could be species dependent, but it could also be due to a voltage drop either along axonal compartments or across the pipette resistance. In the last case, the same voltage drop, i.e., the same right shift of  $I$ - $V$  curves, is also seen for other conductances that are not localized to the axon.

The amplitudes of the  $\text{Na}^+$  currents show a considerable variability and, in some cells (292, 343),  $\text{Na}^+$  current

could not be measured at all. The reason for this variability and occasional lack of  $\text{Na}^+$  currents in ORNs could be that the channels are primarily located in the axonal membrane, which is partially lost during preparation. This is consistent with the finding of Maue and Dionne (227) who were unable to observe single  $\text{Na}^+$  channels on the somata of mouse ORNs. Frings and Lindemann (99) found no effect of TTX when applied to the mucosal surface, whereas it blocked spike generation when applied to the interstitial surface of the mucosa. However, in the mudpuppy,  $\text{Na}^+$  channels were almost equally distributed on somata and dendrites (76).

Interestingly, cytosolic GTP (100  $\mu\text{M}$ ) shifts the inactivation curve of  $g_{\text{Na}}$  to the right (277). This effect is presumably not brought about by GTP itself, because the nonhydrolyzable analog guanosine 5'-O-(3-thiotriphosphate) ( $\text{GTP}\gamma\text{S}$ ) exerts the same effect (277). Under perforated patch, the steady-state inactivation curve for  $g_{\text{Na}}$  overlaps with the inactivation curve measured in the whole cell configuration with  $\text{GTP}\gamma\text{S}$  in the pipette. These results indicate that a constitutively activated G protein maintains low half-inactivation voltages for  $g_{\text{Na}}$  in ORNs. These results open the possibility that  $g_{\text{Na}}$  might be modulated by G protein-coupled receptors.

## B. Voltage-Gated Calcium Currents

High-voltage-activated (HVA)  $\text{Ca}^{2+}$  currents in ORNs have been described in various species (65, 69, 92, 260, 305, 342, 343). The amplitude of this current seems to be relatively small, and in the whole cell configuration of the patch-clamp technique, the maximum current decreases with a time constant of  $\sim 3$  min (305). This phenomenon, often called "washout," has been observed in many preparations and is presumably because of the diffusion between cytosol and patch pipette (14, 278). The HVA  $\text{Ca}^{2+}$  currents activate between  $-40$  and  $-30$  mV and have maximum amplitudes at  $\sim 0$  mV; in some reports, the  $I$ - $V$  relationship appears to be shifted to higher voltages, which may depend on high access resistances (13) or an elevated extracellular  $\text{Ca}^{2+}$  concentration (92, 305, 343). The HVA channels are thus primarily activated during the generation of action potentials, and the resulting  $\text{Ca}^{2+}$  influx presumably contributes to the repolarization by activating  $\text{K}^+$  channels (see sect. III C). Using quantitative ratiometric  $\text{Ca}^{2+}$  imaging with fluo 3 and FuraRed, Schild et al. (309) have shown that the HVA  $\text{Ca}^{2+}$  channels in *Xenopus* ORNs are primarily situated on the soma and the proximal dendrite. The resolution of the method does, however, not exclude that a small portion of  $\text{Ca}^{2+}$  influx could occur in other cellular compartments of ORNs.

In some species, a low-voltage-activated (LVA)  $\text{Ca}^{2+}$  current may also be involved in action potential formation. In the newt, Kawai et al. (153) have reported an

LVA current being half-activated at  $-44$  mV. Although this current is absent in some species [e.g., *Xenopus* (305), Chilean toad (69), and catfish (246)], its description is consistent with Trotier's finding of a TTX-insensitive inward current that could be blocked with  $\text{Co}^{2+}$  in salamander ORNs (343). This current lowers the threshold for action potential firing. It speeds up action potential generation by decreasing the inactivation of the voltage-gated  $g_{\text{Na}}$  during receptor potentials. The LVA  $\text{Ca}^{2+}$  current might therefore be particularly useful in relatively large ORNs that have high capacitances and long membrane time constants.

## C. Potassium Conductances

Repolarization of the action potential is achieved by the activation of various  $\text{K}^+$  channels: first, and above all, by a transient, 4-aminopyridine (4-AP)-sensitive  $\text{K}^+$  current described in squid (217), salamander (92), zebrafish (65), catfish (246), salmon (260), clawed frog (305), and rat (221). This current activates and inactivates rapidly; it activates at potentials more positive than the activation threshold of  $\text{Na}^+$  channels (221, 305), which precludes the traditional role of A currents in spike frequency modulation (64, 120). It rather contributes strongly to the repolarization of action potentials. However, in cultured ORNs of rat, which have a large delayed-rectifier conductance, the fast 4-AP-sensitive current as well as a  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  current were absent (342).

Second,  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  conductances [ $g_{\text{K}(\text{Ca})}$ ] measured in various species (65, 69, 92, 227, 260, 305, 343) seem to contribute to the repolarization and an increase of the cell's impedance, whereby in the mouse, 130-pS channels with voltage-dependent kinetics and 80-pS channels with voltage-insensitive kinetics have been observed (227), presumably corresponding to BK and SK  $\text{K}^+$  channels (190, 196). In agreement with these findings, a fraction of the outward current in *Xenopus* ORNs was blockable by apamin (305), which blocks  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  channels of the SK type (55). Among the various  $\text{Ca}^{2+}$  sources that could potentially activate  $g_{\text{K}(\text{Ca})}$ ,  $\text{Ca}^{2+}$  influx through HVA  $\text{Ca}^{2+}$  channels appears to play the major role because blocking the HVA current (69, 305, 343) or reducing the  $\text{Ca}^{2+}$  flux through HVA channels (92, 260) reduced or abolished the  $\text{Ca}^{2+}$ -dependent conductances. Calcium influx-induced  $\text{Ca}^{2+}$  gradients are very steep, and the diffusion length of free cytosolic  $\text{Ca}^{2+}$  is on the order of 1  $\mu\text{m}$  (143, 259). The  $\text{Ca}^{2+}$ -activated conductances, or at least a major fraction of them, can therefore be supposed to be colocalized with HVA  $\text{Ca}^{2+}$  channels. This is also supported by the fact that the voltage-gated  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  current in ORNs, when activated by voltage steps under voltage clamp, activates simultaneously with the voltage steps (69, 92, 246, 305, 343).

Because most of the HVA  $\text{Ca}^{2+}$  channels seem to be situated on the soma and the proximal dendrite (309), we can conclude that  $g_{\text{K(Ca)}}$  is colocalized with HVA channels at the soma and the proximal dendrite. This is consistent with single-channel measurements in excised patches from olfactory neuronal soma membrane (227); it does, however, not preclude additional  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  channels being localized elsewhere. Bacigalupo and co-workers (248, 249) have reported evidence for a  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  conductance in the transduction compartments of the toad that can be activated by odorants (see sect. VII). Differentiation between conductances involved in action potential generation and others involved in receptor potential generation might turn out to be inappropriate because the same  $g_{\text{Na}}$ ,  $g_{\text{H}}$ , and  $g_{\text{K(Ca)}}$  may be involved in both.

In conclusion, an action potential, initiated by a receptor potential and voltage-gated  $\text{Na}^+$  channels, goes through the voltage range positive to  $-30$  mV, where HVA channels on the soma and the proximal dendrite activate. The resulting  $\text{Ca}^{2+}$  influx activates  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  channels on soma and dendrite which, in concert with fast  $g_{\text{K}}$ , then contribute to the repolarization of the action potential. Maue and Dionne (227) describe two different types of  $g_{\text{K(Ca)}}$ , which opens the possibility that a  $g_{\text{K(Ca)}}$ , in addition to participating in the repolarization, may play an additional role in tuning the refractory period. The third conductance, which may play a role in both repolarization and tuning of the resting and poststimulus impedance, is the slow delayed rectifier-like  $\text{K}^+$  conductance (69, 92, 217, 246, 260, 292, 305, 343).

This particular combination of  $\text{K}^+$  conductances taking part in the repolarization seems to be very similar in all studied species, although an exact quantitative comparison is presently impossible. The general idea, however, appears to be that only very few  $\text{K}^+$  channels are open at rest, resulting in an extremely high resting resistance and a correspondingly high sensitivity. The  $\text{K}^+$  conductances that are open at rest, presumably the inward rectifier and the delayed rectifier, would not be able to repolarize the membrane back to the resting potential because the inward rectifier is closed at depolarized voltages and the delayed rectifier has too small a conductance. Repolarization seems to be done by transiently switching on the fast  $\text{K}^+$  conductance and the fast  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  conductance.

#### D. Summary

As inward currents, ORNs typically express a voltage-gated  $\text{Na}^+$  current and a HVA  $\text{Ca}^{2+}$  current. In addition, some species appear to have an LVA  $\text{Ca}^{2+}$  current. Repolarization of the action potential is achieved by the activation of mainly two  $\text{K}^+$  channels: 1) a transient, 4-AP-sensi-

tive  $\text{K}^+$  current activating at potentials more positive than the activation threshold of  $\text{Na}^+$  channels and 2)  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  conductances  $g_{\text{K(Ca)}}$ . An action potential, initiated by a receptor potential and voltage-gated  $\text{Na}^+$  channels, goes through the voltage range positive to  $-30$  mV where HVA channels on the soma and the proximal dendrite activate. The resulting  $\text{Ca}^{2+}$  influx activates  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  channels on soma and dendrite which, in concert with the inactivation of  $\text{Na}^+$  channels and activation of the fast  $\text{K}^+$  conductance, repolarize the membrane potential during an action potential. The cell's resting impedance and therefore its sensitivity appear to depend primarily on delayed rectifier and inward rectifier channels.

## IV. ODORANT RESPONSES

### A. Recording Methods

The classical recordings from single ORNs were made using either platinum-black electrodes or sharp pipettes (4, 105, 106). Although the sharp electrode recordings suffered from the leak between glass and membrane resulting in short recording durations, extracellular recordings proved useful to determine the differential responsiveness of ORNs to many stimuli (142, 325). The patch-clamp technique with gigaohm seal resistances provided several new methods of recording odorant responses: 1) tight-seal whole cell patch-clamp recordings under voltage-clamp or current-clamp conditions, 2) cell-attached patch-clamp recordings, 3) loose patch recording with a suction electrode (212), 4) perforated patch recordings using pore-forming molecules such as nystatin (76, 217) or gramicidin (361), and 5) sensory cilia attached in loose-clamp (99), tight-seal, or excised configuration (159, 164, 165). The tight-seal whole cell configuration allows voltage clamping of the membrane potential so that activation and inactivation of voltage-gated and odorant-induced conductances could be measured. However, the diffusion of molecules from and into the pipette changes the cell's milieu, resulting in loss of important factors in the cytoplasm. Washout of messengers and modulators can prevent physiological responses, especially when recording  $\text{Ca}^{2+}$  currents or odorant responses. The cell-attached mode allows the measurement of mainly capacitive currents across the patch that are associated with action potentials (219). The perforated-patch configuration prevents the diffusion of large molecules between pipette and cytosol, but the access resistance to the cell is higher than in the whole cell mode. This causes a current-dependent voltage drop across the pipette tip, and, in most patch-clamp amplifiers, the voltage-clamp control circuit is slowed considerably.

In addition to electronic amplifiers, charge-coupled



device (CCD) and laser scanning microscope imaging techniques (296, 306) became available and were used to measure odorant responses, whereby the increase of intracellular  $\text{Ca}^{2+}$  was monitored as an indirect measure of excitation.

## B. Odorant-Induced “Cation” Current

The first manuscripts to present studies of ORNs using the patch-clamp technique were published in 1985 (7) and 1986 (343). Trotier (343) described voltage-gated currents, single channels including the inward rectifier and currents induced by addition of odorants (7  $\mu\text{M}$  isoamylacetate and 10  $\mu\text{M}$  butanol).<sup>1</sup> Although the description of the odorant-induced current was short, it contained some important points that were confirmed later: the reversal potential of the odorant-induced current was  $\sim 0$  mV, the  $I$ - $V$  relationship was almost linear, and there was a considerable delay in the response (in this study  $\sim 2$  s). Trotier (343) cautiously suggested as a working hypothesis that the odor-activated channels were nonselective cation channels modulated by a diffusible cytosolic metabolite. The equilibrium potential for both  $\text{Cl}^-$  and cations was  $\sim 0$  mV in his study.

The history of the discovery of odor-activated currents in vertebrates took three main routes: 1) independently the laboratories of Firestein, Kurahashi, and Gold followed Trotier's working hypothesis and consequently carried out detailed analyses of the odor-activated currents. Kleene and Gesteland (165) discovered that what was believed to be an odor-gated “cation” current was actually a mixture of a  $\text{Ca}^{2+}$ -permeable cation current and a  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  current. These experiments led to the present view of the cAMP-mediated second messenger cascade, which is described in section v. 2) Evidence regarding odorant-induced  $\text{Ca}^{2+}$  and cation currents that were different from the cAMP-gated current did not fit in the model of a cAMP-mediated second messenger cascade. Work from the laboratories of Restrepo, Ache, and Schild as well as from studies in insects (327, 328, 363, 373) indicated that there might be at least one more second messenger pathway mediated by inositol phosphates,  $\text{Ca}^{2+}$ , or guanosine 3',5'-cyclic monophosphate (cGMP). This work is described in sections VI and VII. 3) Several studies reporting odorant-mediated currents did not primarily aim at analyzing elements of the cAMP or inositol trisphosphate ( $\text{InsP}_3$ ) pathway. They rather pointed at the kinetic and biophysical characterization of odorant responses, which are reviewed in the rest of this section.

<sup>1</sup> Whenever odorant-induced responses are discussed, the molecular nature of the odorant used is stated. This is important because, as discussed in section VI A, individual odorants might stimulate different second messenger pathways leading to activation of different conductances.

After Trotier's 1986 paper (343), one study<sup>2</sup> reported the block of the odorant-induced current by amiloride in frog ORNs (98), another reported an odorant-stimulated depolarization that reversed at 2.7 mV (8), and studies from two laboratories presented a detailed characterization of odor-activated currents in ORNs (91, 175, 179, 182). Kurahashi (175) characterized an odorant-gated cation conductance in newt ORNs using *n*-amyl acetate (10 mM) as odorant stimulus (Fig. 2). This current had the following properties: 1) it reversed at  $\sim 0$  mV, the reversal potential shifting by  $\sim 57$  mV upon reducing  $\text{Na}^+$  concentration by a factor of 10; 2) the current was maximum when the odorant was applied at the apical dendrite; 3) the  $I$ - $V$  curve was nonlinear, showing a block of inward current at potentials more negative than  $-30$  mV; 4) its permeabilities for alkali ions were  $P_{\text{Li}}/P_{\text{Na}}/P_{\text{K}}/P_{\text{Rb}}/P_{\text{Cs}} = 1.25:1:0.98:0.84:0.8$ ; 5) removal of  $\text{Ca}^{2+}$  increased the odorant-gated current and prolonged its duration, but did not cause a change in reversal potential; and 6) removal of  $\text{Cl}^-$  did not change the reversal potential. The size of the odor-induced current was maximal when the odor plume was directed to the apical side of the cell. The pipette solutions in these studies contained 5 mM ethylene glycol-bis( $\beta$ -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) so that  $[\text{Ca}^{2+}]_i$  was heavily buffered. In addition, in a separate manuscript, Kurahashi and Shibuya (183) suggested that the inactivation of the odorant-induced current was mediated by  $\text{Ca}^{2+}$  influx through the odor-regulated conductance.

Firestein and Werblin (91) stimulated terrestrial phase salamander ORNs using a mixture of acetophenone, *n*-amyl acetate, cineole, phenylethylamine, and triethylamine (each at a concentration between 0.1 and 1 mM). The reversal potential for cations ( $u_{\text{cat}}$ ) and  $u_{\text{Cl}}$  were both at  $\sim 0$  mV in this study. These authors confirmed and extended Trotier's finding of a purported cation current with respect to the following points: 1) again the  $I$ - $V$  curve of the odor-activated currents was linear with a reversal potential of  $\sim 0$  mV, 2) the delay of the odorant response was assessed more accurately (140–570 ms), 3) a dose-response curve extending from  $\sim 10^{-5}$  to  $10^{-4}$  M for a mixture of stimuli was given, and 4) the odor response was shown to be cooperative with a Hill coefficient of 2.7. Any of the steps between binding of the odorant and flow of ions through odorant-activated channels could thus be cooperative, leading to a sigmoidal input-output (i.e., concentration-current) relationship (see also Ref. 87). In a later study, Firestein et al. (90) confirmed that the odorant sensitivity is restricted to the cilia and possibly the distal dendrite. They also found that the latency of the response was relatively independent of the stimulus concentration ( $10^{-5}$  and  $10^{-6}$  M).

<sup>2</sup> The odorants used in this study were cineole, amyl acetate, and isobutylmethoxyppyrazine at a concentration of 0.5  $\mu\text{M}$ .

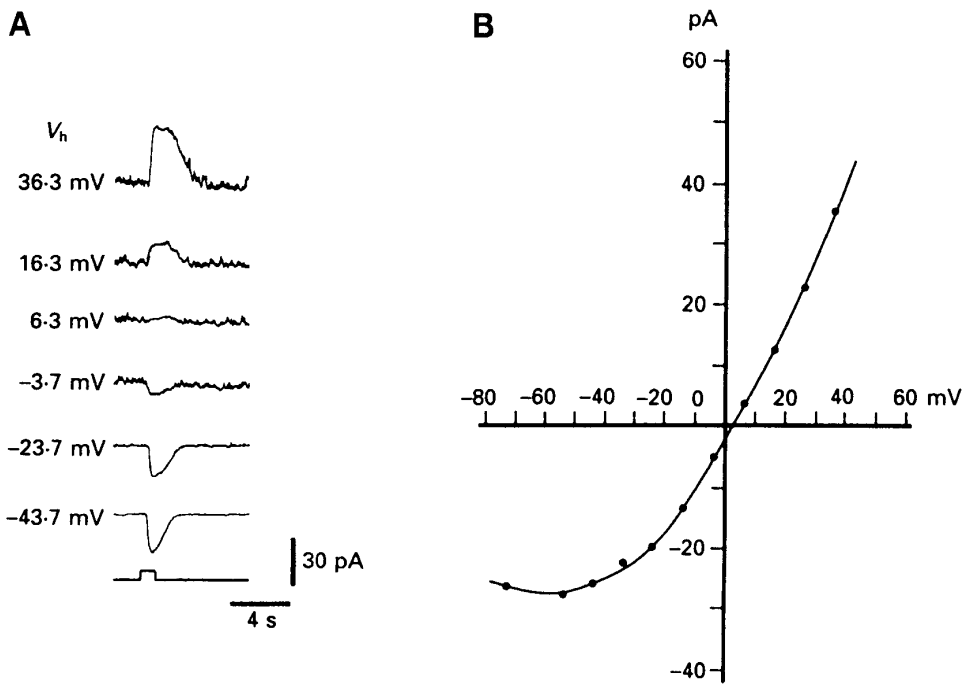


FIG. 2. A: odorant-induced currents recorded at various holding voltages ( $V_h$ ; indicated to left of each current trace) from a newt (*Cynops pyrrhogaster*) olfactory receptor neuron. Current traces were arbitrarily shifted. Bottom trace indicates timing of *n*-amyl acetate (10 mM) application from a glass pipette positioned  $<20 \mu\text{m}$  away from apical dendrite. B: voltage dependence of odorant-induced current; replotted from A and additional records from same cell. [Modified from Kurahashi (175).]

The spatial distribution of odorant sensitivity and of the odorant-induced current was carefully examined in a series of experiments in salamander ORNs by Lowe and Gold (212). Using an odorant mixture containing 2-hexylpyridine, isoamyl acetate, acetophenone, and cineole, these investigators determined that the odorant response increased approximately linearly as a function of the fraction of the ciliary bundle that was exposed to the odorant. No further increase in responsivity was found upon stimulation of the entire ciliary bundle and a fraction of the dendrite. This indicated that olfactory receptor sites are uniformly distributed along the length of the cilium and are excluded from the dendrite. In addition, the odorant-induced current was found to be localized to the cilia, and the resting  $\text{K}^+$  conductance was found to be much lower in the ciliary membrane than in the dendritic membrane. The pipette contained a pseudointracellular solution with 0.1 mM EGTA, pH 7.2, providing little buffering of intracellular  $\text{Ca}^{2+}$ .

Obviously, in a sensory system that can possibly detect single molecules (215, 238), concentrations in the range between  $10^{-5}$  and  $10^{-6}$  M are high. However, the physical chemistry of lipophilic odorants in water is by no means trivial, and it has to be kept in mind that in the experiments described, the odorants were applied in the absence of mucus or odor-binding proteins, i.e., the perireceptor microenvironment (107, 139, 224, 235, 271) was far from physiological. The experiments by Frings and Lindemann (99), who recorded from single cilia of ORNs in an at least partly intact epithelium, are interesting in this respect because they recorded an increase in action potential rate upon application of cineole (dissolved in

ethanol), whereby 9, 5, and 3 of 22 responding cells responded to odorant concentrations of 1, 10, and 100 pM, respectively (99).

### C. Optical Recordings of Odorant Responses

Optical recordings of odorant-induced changes in  $[\text{Ca}^{2+}]_i$  with CCD cameras or laser scanning microscopes have the advantage of spatial resolution, and they are minimally invasive, although the endogenous  $\text{Ca}^{2+}$  buffering capacity of the cells (158) is increased by adding the reporter dye (118). Optical recording of  $[\text{Ca}^{2+}]_i$  was first used by Restrepo and co-workers (288, 290) to monitor odorant responsiveness of catfish ORNs. These and subsequent studies determined that odorants elicit increases in intracellular  $\text{Ca}^{2+}$  (16, 145, 192, 257, 288, 291, 292, 302, 337) and that removal of extracellular  $\text{Ca}^{2+}$  abolished the odorant-induced increases in  $[\text{Ca}^{2+}]_i$  in ORNs, suggesting that the increase was due to influx of  $\text{Ca}^{2+}$  through the plasma membrane (16, 288, 291, 292, 302, 337). However, some olfactory neurons from bullfrog (*Rana catesbeiana*) were found to respond even in the absence of extracellular  $\text{Ca}^{2+}$  (302). Imaging experiments determined that shortly after stimulation, and in some ORNs even after prolonged stimulation, the increases in  $[\text{Ca}^{2+}]_i$  took place at the apical end of the cell, suggesting that the source of  $\text{Ca}^{2+}$  was at the apical compartments of the cell (16, 257, 291, 302, 337). Interestingly, recent measurements with confocal microscopy in salamander (*A. tigrinum*) ORNs has demonstrated odor-induced changes in  $[\text{Ca}^{2+}]_i$  in individual olfactory cilia (192). The increase in

$[Ca^{2+}]_i$  in the cilia is rapid and transient and contrasts with a much slower and prolonged increase in  $[Ca^{2+}]_i$  in the cell body.

Imaging of  $[Ca^{2+}]_i$  has also been used to study channels and transporters involved in  $[Ca^{2+}]_i$  regulation in ORNs and the regulation of these channels and transporters by second messengers. Thus it has been possible to localize the HVA  $Ca^{2+}$  current in ORNs (309), the  $Na^+/Ca^{2+}$  antiport (136), and  $InsP_3$ -activated,  $Ca^{2+}$ -permeable channels of the plasma membrane of ORNs (145, 311).

Studies of odorant responsiveness utilizing  $[Ca^{2+}]_i$  imaging techniques have provided information on the odor specificity of neighboring ORNs. Takebayashi and co-workers (122, 303) imaged a partly dissociated preparation of mouse ORNs in which the cells retained their original spatial relationships. With the use of three different odorants in one study (122) and a series of aliphatic odorants in another study (303), the responsiveness of ORNs was estimated from the odor-induced increase in  $[Ca^{2+}]_i$ . These authors were able to demonstrate that cells with a similar odor responsivity were located next to cells with a different odor responsivity. The distribution patterns were, however, not consistent with a completely random distribution.

Finally, optical recording with voltage-sensitive dyes (VSDs) has been used to determine neuronal activity in the olfactory system (60, 149). This technique has the advantage that the average activity in a volume of tissue can be determined upon stimulation with many odorants (150). Staining of the olfactory epithelium with VSDs could be used to study signal transduction in ORNs, which would be particularly useful in resolving questions about signaling between neighboring ORNs. However, the use of VSDs in the olfactory epithelium at high magnification has not been reported in refereed publications. Voltage-sensitive dyes have been used to record distributed activity within the olfactory epithelium and the olfactory bulb at low magnification (cf. Refs. 59, 61, 62, 155, 156, 356). These experiments have provided an important contribution of our understanding of patterns of responsivity to odors in the olfactory epithelium and form a basis for understanding olfactory quality coding. Studies of distributed patterns of responsivity with VSDs are not reviewed in detail here, and the reader is referred to recent reviews (60, 149).

#### D. Diversity of Transduction Mechanisms

Some studies of odorant responsivity suggest mediation by cAMP or  $InsP_3$  on the basis of pharmacology, genetic manipulations, ionic dependence, or biochemical measurements. Responses mediated by opening of cAMP-gated channels have been studied in detail and are described in section v. The evidence for  $InsP_3$  as a second

messenger in the signaling of ORNs is still controversial and is delineated in section vi. In addition, a number of manuscripts suggest the existence of multiple olfactory transduction pathways but do not reveal the nature of the second messenger mediating the responses, whereas other experiments show responses to odorants that cannot currently be explained within the context of the models proposed for the cAMP and  $InsP_3$  pathways. These studies are discussed below.

##### 1. $[Ca^{2+}]_i$ responses to odorants are differentially affected by diltiazem and neomycin

In several studies, odor-induced increases in  $[Ca^{2+}]_i$  have been used as an indirect marker for cell activity to study the pharmacology of the second messenger pathways that participate in olfactory transduction. Human and rat ORNs responded with  $Ca^{2+}$  increases of 20–200 nM upon application of a mixture of hedione, geraniol, phenylethylalcohol, citralva, citronella, eugenol, and menthone (100  $\mu$ M each), and this response could be blocked by *L-cis*-diltiazem (291, 292), which blocks the olfactory cyclic nucleotide-gated channel (101, 170). Tareilus et al. (337) imaged  $[Ca^{2+}]_i$  in rat ORNs using a laser-scanning microscope and the fluorescent  $Ca^{2+}$  indicators fluo 3 and FuraRed, a method introduced by Lipp and Niggli in myocytes (203) and by Schild et al. in neurons (309). Using two mixtures of odorants [*mixture I*: citralva, eugenol, hedione (1  $\mu$ M each); *mixture II*, ethylvanilin, lilial, lylal (1  $\mu$ M each)], they demonstrated that the  $[Ca^{2+}]_i$  response to *mixture I* was blocked by *L-cis*-diltiazem but not by neomycin, an inhibitor of phospholipase C as well as of certain  $Ca^{2+}$  channels (114, 126, 207, 330). In contrast, the response to *mixture II* could be blocked by neomycin but not *L-cis*-diltiazem. A similar differential effect of neomycin and *L-cis*-diltiazem on odorant responses has also been described in human olfactory neurons (282) and in human olfactory neuroblastoma cells (110). Although these experiments cannot definitively determine the identity of the two pathways because the drugs used are known to interact with multiple targets, they indicate that different odorants stimulate pharmacologically distinct transduction pathways.

##### 2. Inhibitory responses to odorants

Inhibitory responses, i.e., those that elicit suppression of the basal rate of firing of action potentials, were first reported by Gesteland (104) and later by other investigators (these reports were discussed controversially, see Ref. 106 for review). In the last decade, more laboratories have reported inhibitory responses, and in some species, the underlying mechanism is beginning to be understood. In mouse, Maue and Dionne (227) reported inhibitory re-

sponses in on-cell tight patches in isolated ORNs. In the mudpuppy, application of taurine induced increases or decreases of a  $\text{Cl}^-$  conductance (73, 75, 76). In the squid, L-dopa, dopamine, and squid ink inhibited spiking activity presumably by activating  $\text{Cl}^-$  or  $\text{K}^+$  channels (217). Inhibition of spiking activity cannot be explained by the cAMP-mediated pathway, except when inhibition is an artifact based on choosing a low  $u_{\text{Cl}}$  near the resting potential. Inhibition of spiking activity by amino acid odorants has been clearly demonstrated in a large percent of channel catfish ORNs by Kang and Caprio (142). Because these authors used extracellular recordings, the intracellular milieu of the ORNs was unaffected so that the inhibition shown in this study clearly points to a transduction mechanism that is different from the cAMP-mediated transduction cascade. A similar result is found in the lobster, where odorants elicit either depolarization, mediated by opening of an  $\text{InsP}_3$ -gated nonspecific cation channel, or hyperpolarization, mediated by opening of a cAMP-gated  $\text{K}^+$  channel (27, 80, 116, 228, 229, 240, 241, 313). Interestingly, Zhainazarov and Ache (360) have speculated that in lobster ORNs, opening of the  $\text{InsP}_3$ -gated cation channel might lead to a change in intracellular  $\text{Na}^+$ , leading to opening of a  $\text{Na}^+$ -activated nonspecific cation conductance. Another inhibitory mechanism has been described in Chilean toad where Bacigalupo and co-workers (16, 248, 249) have described an odorant-induced increase in  $\text{K}^+$  conductance (discussed in detail in sect. VII). In the newt, Kawai et al. (154) find suppression of all voltage-gated currents at high odorant concentrations. They conclude that at high concentrations of odorants action potential firing is suppressed because of nonspecific blockage of voltage-dependent currents. Therefore, it is clear that at least in some species there are dual transduction pathways leading to excitation or suppression of action potential firing in ORNs.

### 3. Evidence for further transduction pathways

In ORNs of the Atlantic salmon, which are believed to be sensitive to amino acids (331), a metabotropic glutamate receptor linked to phospholipase C has been shown by Pang et al. (269). Whether glutamate induces an inhibitory or an excitatory response in these cells is not yet established. However, the idea is intriguing that receptors that are present in CNS neurons may also serve as olfactory receptor proteins.

In *Caenorhabditis elegans*, mutation of cyclic nucleotide-gated channel subunits affects responsivity to the odorants benzaldehyde, 2-butanone, and isoamyl alcohol, but not to diacetyl, pyrazine, and trimethylthiazole (63, 172). The nature of the second messenger pathway mediating transduction for the second group of odorants is not known.

Excitatory mechanisms different from the cAMP- or  $\text{InsP}_3$ -mediated pathways have also been described in several species. One mechanism that does not appear to be consistent with the cAMP- or  $\text{InsP}_3$ -mediated transduction cascades is the odorant-induced blockage of  $\text{K}^+$  channels (75). A similar mechanism is known in taste receptor cells where natural sweet stimuli cause the phosphorylation and the blockage of  $\text{K}^+$  channels (15, 25, 66, 201). Another observation that cannot be explained within the context of the proposed models for mediation of olfactory transduction by cAMP and  $\text{InsP}_3$  that predict odorant-induced increases in  $[\text{Ca}^{2+}]_i$  is an odorant-induced decrease in  $[\text{Ca}^{2+}]_i$  recorded in ORNs from human (282) and catfish (288). Particularly interesting is the finding of ORNs that express a membrane-bound guanylyl cyclase and a cGMP-stimulated phosphodiesterase but neither an adenylyl cyclase III nor a cAMP-dependent phosphodiesterase (135). These ORNs might respond to odorants by directly increasing cGMP and gating the cGMP/cAMP-dependent cation conductance. Furthermore, it is important to indicate that ion channels are not necessarily the only targets of potential transduction cascades. For example, in *Paramecium*, the attractive stimulus acetate elicits a calmodulin-mediated stimulation of a  $\text{Ca}^{2+}$ -ATPase which hyperpolarizes the cell membrane causing an increase in swimming speed (355). In cilia of the Atlantic salmon, a  $\text{Ca}^{2+}$ - $\text{Mg}^{2+}$ -ATPase and a  $\text{Na}^+$ - $\text{K}^+$ -ATPase have been shown (209, 211), both of which would also hyperpolarize the neuron. In summary, there are several candidates for new olfactory transduction mechanisms that must be addressed by future studies.

### 4. Are ORNs modulated by the autonomic nervous system?

A further interesting point within the context of diversity in transduction mechanisms is the possible influence of the autonomic nervous system on olfactory transduction pathways. Some neurotransmitter receptor antagonists such as propranolol, a  $\beta$ -adrenergic antagonist and a serotonin ( $5\text{-HT}_1$ ) agonist, and the muscarinic antagonist atropine can block odorant responses in isolated ORNs via an unknown mechanism (88). In addition, neurotransmitter agonists and antagonists can act at sites other than olfactory receptors. Frings (96) showed that both serotonin ( $5\text{-HT}$ ) and the muscarinic agonist carbachol increased the spiking rate of ORNs by activating the adenylyl cyclase via phosphokinase C (96). Muscarinic receptors have been shown in the olfactory epithelium (117), and acetylcholine as well as  $5\text{-HT}$  may be contained in vesicles of nerve endings and varicosities of the ophthalmic branch of the trigeminal nerve (364). The pathway studied by Frings (96) could therefore be the intracellular cross-talk between autonomic modulation of ORNs and

odorant responses. The autonomic influence on discharge rates of ORNs had been shown by antidromic stimulation of the ophthalmic branch (32) and by direct application of acetylcholine and substance P to the epithelium (33). Moreover, the dopamine D<sub>2</sub> agonist bromocriptine can inhibit adenylyl cyclase activity in ORNs (225). However, the localization of the D<sub>2</sub> receptor, the G protein it couples to, and the adenylyl cyclase has not yet been clearly determined, although the authors speculate the D<sub>2</sub> receptors could be situated in the nerve terminals (225). Hence, this mechanism would presumably not interfere directly with odor transduction processes, but it could be involved in the organization of the synapses between primary nerve fibers and projecting neurons in the olfactory bulb (263, 323).

### 5. Odorant-gated channels

It is now generally believed that olfactory signal transduction is mediated by second messengers (see sects. v–vii). However, some evidence has implicated ion channels directly gated by odorants. These were measured in membrane vesicles from olfactory tissue homogenates incorporated into artificial lipid bilayers (85, 186, 351, 377). Two different odorant-activated channels have been described. Vodyanoy and Murphy (351) found a 62- to 65-pS K<sup>+</sup>-selective channel from rat activated by diethylsulfide and/or (–)-carvone that is blocked by 125 nM 4-AP, and Labarca et al. (186) detected a 35-pS nonspecific cation channel from frog activated by 3-isobutyl-2-methoxypyrazine. Odorants induced increases in membrane conductance at nanomolar concentrations, and the response was concentration dependent. Neither ATP nor GTP was required for odor responsiveness. In a separate study, Zviman and Tien (377) found that odorant-gated conductances were activated in a stereospecific manner by structurally related odorants (diethylsulfide, thiophene, and diethanosulfide). These pieces of preliminary evidence suggested the existence of olfactory transduction pathways that function by direct activation of ligand-gated ion channels.

### E. Responses of Olfactory Neurons in Culture

It has proven difficult to culture functionally responsive ORNs. The only report of odorant-induced changes in electrical activity is from cultured ORNs of rat bearing multiple long processes that showed odorant-induced depolarizations in conjunction with an increase in conductance. Interestingly, these cells also displayed a second type of odorant-activated current that resembled very much synaptic currents (275). Others have reported odorant-induced changes in second messenger formation and/or odorant-induced changes in [Ca<sup>2+</sup>]<sub>i</sub> in cultured olfac-

tory neuroblasts. Long-term cell cultures of neuroblasts from human fetal olfactory epithelium and olfactory neuronal cell cultures from rat embryo responded to stimulation by odorants with biochemically measured increases in cAMP or InsP<sub>3</sub> formation (297, 347), and human olfactory neuroblastoma cells (110) and neuronal cells cultured from adult human olfactory epithelium (354) have been shown to respond to odors with increases in intracellular Ca<sup>2+</sup>.

Cell cultures of olfactory neuroblasts have also been used in the study of olfactory mechanisms in invertebrates. Cultured ORNs of lobster responded to odorants even before they had developed processes (83), suggesting that these cells insert all transduction components in the soma membrane during their development. Cultured ORNs of the silkworm *Manduca sexta* responded to a pheromone (synthetic bombycal) with a sequence of inward currents. The early component of the response to the pheromone was a Ca<sup>2+</sup> current that has been suggested to be regulated by InsP<sub>3</sub> (328). This current was inhibited by Ni<sup>2+</sup> and did not appear in the absence of Ca<sup>2+</sup> or Ba<sup>2+</sup> in the bath. It was followed by a slower inwardly rectifying current component with a reversal potential near 0 mV. The nonspecific current had the characteristics of a Ca<sup>2+</sup>-dependent nonspecific cation current described in insect ORNs (327). Finally, a third sustained component reversed near 0 mV and was proposed to be a protein kinase C-dependent cation current (327, 328).

### F. Responses of Vomeronasal Neurons

Responses of single vomeronasal ORNs to odorants or pheromones have as yet not been reported. However, Trotier et al. (345) and Liman and Corey (200) have measured voltage-gated currents in microvillous vomeronasal neurons using the patch-clamp technique. Interestingly, Liman and Corey (200) report a lack of a response upon dialysis into the cytosol of vomeronasal receptor neurons with cAMP (200), whereas Taniguchi et al. (335, 336) report current responses upon dialysis of vomeronasal receptor cells of the turtle with cAMP, cGMP, or InsP<sub>3</sub>. Furthermore, Okamoto et al. (265) have shown a forskolin-induced increase of adenylyl cyclase in turtle vomeronasal receptor cells, but odorants that increase the activity of this enzyme in ORNs of other species had no effect. Hence, there appear to be some dissimilarities between the receptor cells in the main olfactory epithelium and those in the vomeronasal organ (197). This has been confirmed by Dulac and Axel's (77) finding that a family of putative receptor genes of the rat vomeronasal organ is unrelated to the receptors expressed in the rat main olfactory epithelium, and by studies from Berghard et al. (24) indicating that, unlike ORNs, vomeronasal neurons do not express mRNA for the G protein G<sub>olf</sub>, for subunit

1 of the olfactory cAMP-gated channel ( $g_{cn1}$ ) and for adenylyl cyclase III.<sup>3</sup> Vomeronasal neurons do express mRNA encoding for the second subunit of the olfactory cAMP-gated channel ( $g_{cn2}$ ) (24). In the absence of  $g_{cn1}$ ,  $g_{cn2}$  does not open in response to increases in the concentration of cyclic nucleotides, but does open upon exposure to nitric oxide (NO) (44). A channel with characteristics consistent with those of  $g_{cn2}$  opens in response to NO in patches excised from rat vomeronasal neurons (44) (see Ref. 197 for review).

## G. Summary

The first reliable measurements of odor-induced receptor potentials and generator currents were obtained using the whole cell configuration of the patch-clamp technique. Some odorants led to an intracellular increase of the second messenger cAMP, which directly and indirectly activated two conductances that were permeable for cations and  $Cl^-$  (see sect. v). Other odorants induce an increase of the second messenger  $InsP_3$  (see sect. vi). Optical recordings showed that the excitation of an ORN is often accompanied by an increase in  $[Ca^{2+}]_i$  in certain cells even in the absence of extracellular  $Ca^{2+}$ . The evidence that reaches beyond the cAMP- and  $InsP_3$ -mediated olfactory transduction pathways is clearly preliminary at present, but it suggests diversity of olfactory transduction processes, e.g., the existence of inhibitory pathways, the generation of receptor potentials via activation or blockage of  $K^+$  channels, and the activation of the classical cAMP/cGMP-gated channels by cGMP generated by a membrane-bound guanylyl cyclase that acts both as receptor and second messenger-producing enzyme.

## V. ADENOSINE 3',5'-CYCLIC MONOPHOSPHATE-MEDIATED SIGNALING IN OLFATORY CELLS

### A. Olfactory Receptors, $G_{olf}$ , and cAMP Formation

It is now generally believed that most odorants bind to seven transmembrane (TM) receptors that couple to G proteins resulting in second messenger formation (40, 46, 51, 52, 181, 189, 232, 262, 268, 274, 281, 283, 322, 324) (Fig.

<sup>3</sup> Because there is no consistent nomenclature for the two subunits of the olfactory cAMP-gated channel, we have chosen to use nomenclature consistent with the rest of the review for the two subunits. We use  $g_{cn1}$  for the first subunit (72, 111, 218) and  $g_{cn2}$  for the second subunit (35, 199). In different manuscripts,  $g_{cn1}$  is labeled either rOCNC1 or  $\alpha$ , whereas  $g_{cn2}$  is referred to as rOCNC2 or  $\beta$  (cf. Refs. 35, 44, 199).

3). A G protein called  $G_{olf}$ , which was first cloned on the basis of enriched expression in the olfactory system, is localized to the olfactory cilia (132, 234). Virtually identical G proteins have also been found in the striatum (119) and tissues outside the CNS such as the pancreas (365). Although no functional data are available directly implicating  $G_{olf}$  in olfactory transduction, it is most likely that  $G_{olf}$  couples certain olfactory receptors to cAMP formation by mediating stimulation of type III adenylyl cyclase (17). Accordingly, biochemical studies of adenylyl cyclase activity and measurements of cAMP formation in isolated olfactory cilia implicated a G protein-mediated increase in cAMP in olfactory transduction (267, 326).<sup>4</sup> However, some potent odor stimuli failed to activate adenylyl cyclase (see sect. vi) (49, 326).

These findings were obtained with conventional biochemical methods and were confirmed and extended in experiments in the subsecond time range utilizing stopped flow methods that demonstrated rapid transient stimulation of either cAMP or  $InsP_3$  formation in isolated olfactory cilia from rat and catfish (28, 37, 38, 287). It must be emphasized that a strict comparison of time courses between these biochemical measurements and measurements of odor-activated currents in intact olfactory neurons is not meaningful because the biochemical measurements were performed on detergent-treated fragments of olfactory cilia where the reactants and products are free to diffuse, whereas in the intact cell, diffusion within the cilia is restricted to a small volume. However, the rapid stimulation of second messenger formation by odorants in isolated olfactory cilia, with a time to peak ranging from 25 to 50 ms, indicates that the biochemical reactions can be fast enough to mediate olfactory responses, which have latencies of 50–500 ms in intact cells (cf. Refs. 90, 175, 213, 246). In addition, biochemical measurements showed that stimulation of both second messenger pathways by odorants was dependent on guanine nucleotides (28, 208, 210, 287) and that the cAMP and  $InsP_3$  pathways were differentially sensitive to bacterial toxins (28), implicating different G proteins in mediating the cAMP and  $InsP_3$  responses.

### B. Olfactory Cyclic Nucleotide-Gated Conductance

A cAMP-gated channel ( $g_{cn}$ ), analogous to the cGMP-gated channel of vertebrate photoreceptors (5), was first

<sup>4</sup> The term *isolated olfactory cilia* is somewhat misleading in the sense that this preparation contains not only cilia from olfactory receptor neurons but also microvilli from sustentacular cells and, depending on the care taken during the dissection, cilia from respiratory cells (11, 34, 57). This fact must be taken into account in evaluating biochemical and electrophysiological experiments utilizing isolated olfactory cilia. Furthermore, it has to be kept in mind that isolated cilia are usually obtained by a "Ca<sup>2+</sup> shock," i.e., a sudden increase of the extracellular Ca<sup>2+</sup> concentration, whose effects on olfactory transduction remain to be studied in detail.

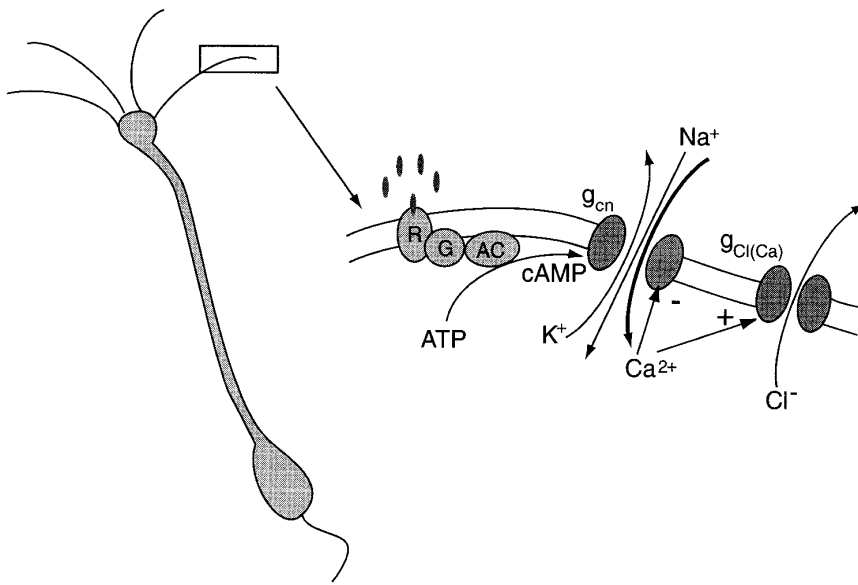


FIG. 3. Schematic diagram of cAMP-mediated transduction cascade of an olfactory neuron. Olfactory receptor neuron consists of at least 5 compartments (*left*): axon, soma, dendrite, dendritic knob, and cilia. Elements of transduction cascade reside in cilia membrane. A simplified model of cAMP-mediated signal pathway is shown on *right*. R, receptor; G, G protein; AC, adenylyl cyclase;  $g_{cn}$ , cyclic nucleotide-activated conductance;  $g_{Cl(Ca)}$ , intracellular  $Ca^{2+}$ -activated  $Cl^-$  conductance. For simplicity, intracellular  $Ca^{2+}$ -dependent phosphodiesterase that transforms cAMP to AMP (29, 30), protein kinase A (26), and receptor kinase responsible for receptor desensitization (68, 312) are left out.

detected in excised patches from olfactory cilia of toad (*Bufo marinus*) ORN by Nakamura and Gold (255). These authors reported activation of nonspecific cation channels by cAMP and cGMP in excised patches from olfactory cilia membrane. The cAMP-gated channels were found to be permeable for  $Ca^{2+}$ , and the authors suggested that voltage-dependent block by extracellular  $Ca^{2+}$  and  $Mg^{2+}$  elicited outward rectification in the  $I$ - $V$  relationship. These observations were confirmed and extended by subsequent work from various laboratories (cf. Refs. 35, 49, 72, 86, 93, 97, 100, 101, 111, 133, 164, 170, 176–178, 181, 183, 199, 213, 218, 223, 245, 273, 332, 370, 371, 374, 376). Similar channels have been found in various other tissues including retina, aorta, and testis (151, 152, 353, 357). Some odorants appear to directly suppress the cAMP-gated conductance  $g_{cn}$  via an unknown mechanism (180).

The evidence in support of the mediatory role of cAMP-gated channels in olfactory transduction is conclusive. Lowe and Gold (213) have shown that odorant-stimulated currents are, except for a difference in latency, identical to currents induced by photorelease of cAMP (a mixture of odorants was used in this study including cineole, isoamylacetate, and 2-hexylpyridine) (213) (Fig. 4). Importantly, however, a large fraction of the cAMP-induced current is carried by  $Cl^-$  (see sect. vC). In addition, on-cell patch measurements at the olfactory knob from Zufall and co-workers (93) indicate that the channels opening in response to odorant stimulation are identical to cAMP-gated channels measured in excised patches in the inside-out configuration (the odorant mixture used contained amylacetate, acetophenone, and cineole) (93). Blockers of cAMP-gated channels including amiloride, cadmium, and *L-cis*-diltiazem inhibit odorant responses (86, 98, 99, 273, 291, 292), and odorant responses and responses to increased cytosolic cAMP levels display the same ionic

dependence (86, 97, 175, 176). In addition, a cAMP-gated channel-deficient transgenic mouse is anosmic to all odorants tested as assessed by electroolfactogram (EOG) measurements (50), and a mutation in a cyclic nucleotide-gated channel subunit in *C. elegans* disrupts responsivity to selected odorants (63, 172).

The cAMP-gated channels have been shown to be localized mainly to olfactory cilia where they can reach a density of 205–2,400 channels/ $\mu m^2$  (166, 177, 178, 371), although channels are found at lower densities (0.2–25 channels/ $\mu m^2$ ) on the soma of the receptor neurons (169, 177, 178, 332) (some of these values may be overestimates due to the assumption that the patch area is equal to the area of the tip of the pipette, see Refs. 166, 301). Addition of low concentrations of cAMP to excised patches of ORN soma membrane in the nominal absence of divalent cations in the pipette elicits current fluctuations typical of single-channel openings. The unitary conductance has been reported to be in the range from 12 to 55 pS depending on the species (Table 2), and in some species a subconductance level is present (111, 178). The apparent binding constant for cGMP is slightly smaller than the binding constant for cAMP, except in the salamander where the apparent binding constant for cGMP is five times smaller than the binding constant for cAMP (compare native channels in Table 2). The apparent binding constants for cAMP and cGMP decrease slightly when the holding potential is shifted from negative to positive values (35, 101, 169, 199). Interestingly, the dose-response relationship for cAMP-gated channels from all species studied, except for the carp, has a Hill coefficient larger than unity, indicating that multiple cyclic nucleotide molecules interact with a single cAMP-gated channel in a cooperative manner (Table 2).

Two subunits of the olfactory cAMP-gated channel

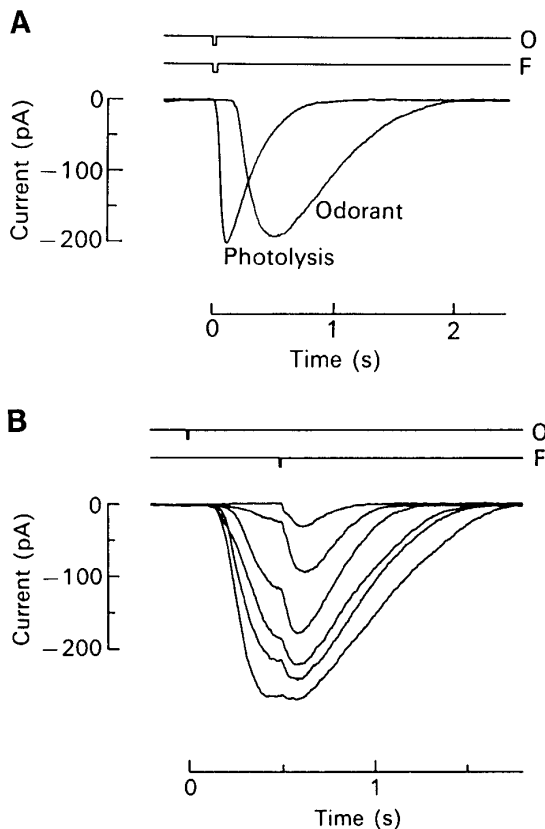


FIG. 4. *A*: whole cell current responses to photolysis of caged cAMP and odorant stimulation in a tiger salamander (*Ambystoma tigrinum*) olfactory receptor neuron. Odorant stimulus (O; duration, 30 ms; pressure, 100 kPa) and flash (F; duration, 40 ms; log attenuation, 0.54) both began at zero on time axis. Notice that response to flash is immediate, whereas response to odorant has a significant delay. *B*: summation of odorant and photolysis responses. Odorant stimulus (O) was followed by a flash (F) that was limited to cilia and timed to coincide approximately with peak of odorant response. Intensity and duration of flash were fixed (log attenuation, 0.54; duration, 10 ms), whereas amplitude of odorant response was varied by adjusting ejection pressure applied to stimulus micropipette (7–175 kPa; duration, 20 ms). Notice that response to flash is not simply additive, but increases and subsequently decreases in magnitude as odorant concentration is increased. This behavior is expected if both stimuli activate cAMP-gated channel at level of increases in cAMP concentration. Nonlinear behavior of flash response is predicted from Hill equation, which describes cAMP concentration dependence of  $g_{cn}$  (see Ref. 213 for details). [From Lowe and Gold (213).]

sharing a considerable degree of homology have been cloned to date (22, 35, 63, 72, 111, 172, 199, 218). The first subunit ( $g_{cn1}$ ) (see footnote 3) can form functional cAMP-gated channels when expressed in a heterologous cell system (72, 111, 218), whereas the expression of the second subunit ( $g_{cn2}$ ) alone does not result in expression of functional channels (35, 199). When  $g_{cn1}$  is expressed alone, the affinity for cAMP is two orders of magnitude lower than the affinity of the native channel (Table 2). However, when  $g_{cn1}$  and  $g_{cn2}$  are coexpressed, the affinity for cAMP increases to within the same order of magnitude of the affinity for the native cAMP-gated channel. On the

basis of the similarity in affinity for cyclic nucleotides, and because of similarities in single-channel properties, it has been suggested that the native cAMP-gated channel is a heterooligomeric  $g_{cn1}/g_{cn2}$  protein complex (35, 199). It is important to state, however, that the native channel has not been definitively shown to be a  $g_{cn1}/g_{cn2}$  heterooligomer.

The cAMP-gated channels are permeable for monovalent alkali cations including  $Na^+$  and  $K^+$  and for divalent cations including  $Ca^{2+}$  (22, 72, 101, 170, 176, 179). The permeabilities of organic cations through these channels have been investigated by Balasubramanian et al. (19). The cAMP-gated channels have been reported to be inhibited by amiloride (101, 162, 273, 332) and dichlorobenzamil (160, 169, 170), the  $Ca^{2+}$  channel blockers cadmium (193), nifedipine (370), *L-cis*-diltiazem (101, 160, 170), *D-cis*-diltiazem (101), and D-600 (101); the calmodulin antagonists *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide, calmidazolium, and trifluoperazine (162); the guanylate cyclase inhibitor LY-83583 (195); and Rp-cAMPS, a structural analog of cAMP (42). The use of these blockers to classify odor responsivity must be exercised with care because of known effects of the drugs on other conductances or enzymes and because of the fact that the action of these drugs has only been reported for certain species.

The conductance of cAMP-gated channels is modulated by both  $[Ca^{2+}]_i$  and extracellular  $Ca^{2+}$  concentration ( $[Ca^{2+}]_o$ ) (18, 56, 102, 161, 163, 173, 175, 206, 223, 255, 370, 376). Increases in  $[Ca^{2+}]_i$  inhibit the cAMP-gated channel. This was first described by Zufall et al. (376) and interpreted as a direct allosteric effect of  $Ca^{2+}$  on the  $g_{cn}$  channels (376). The inhibitory effects of  $[Ca^{2+}]_i$  and intracellular  $Mg^{2+}$  concentration on  $g_{cn}$  channels were then analyzed in more detail (161, 223) and are now believed to be mediated by calmodulin (56, 206) and at least another unidentified endogenous factor (18). By stimulating newt ORNs with caged cAMP and odorants, Kurahashi and Menini (181) have shown that short-term adaptation occurs downstream of the olfactory receptors and provide a calculation showing that the kinetics of adaptation are consistent with mediation by modulation of the cAMP-gated channel by  $[Ca^{2+}]_i$ /calmodulin (181).

The effect of  $[Ca^{2+}]_o$  on  $g_{cn}$  is a permeation block;  $Ca^{2+}$  reduces the permeabilities for other ions while passing through the pore (102) so that, under physiological conditions, i.e., with  $[Ca^{2+}]_o$  in the low millimolar range, the current through these channels is almost exclusively carried by  $Ca^{2+}$ . They are thus  $Ca^{2+}$  channels. As all  $Ca^{2+}$  channels, they have an increasing permeability for other cations when external  $Ca^{2+}$  is removed. Under resting conditions, the  $Ca^{2+}$  influx through these channels can lead to a standing  $Ca^{2+}$  gradient in ORNs (204), but the current amplitude is presumably in the range of a few picoamperes (102). With a membrane resistance of  $\sim 10$  G $\Omega$ , this current could cause sufficient membrane depo-



TABLE 2. Properties of olfactory cAMP-gated channels from various species

Species	Conductance, pS	cAMP			cGMP			Reference No.
		$K_{1/2}$ , $\mu\text{M}$	$u_m$ , mV	$n$	$K_{1/2}$ , $\mu\text{M}$	$u_m$ , mV	$n$	
Rat								
Native	12–15	2.5	+50	1.8	1.0	+50	1.3	101
$g_{cn1}$	35, 48	68	+40	2.0	2.4	+40	2.0	72, 199, 35
		59	+80	2.3	2.2	+80	2.8	
		47	+60	2.6	1.4	+60	2.4	
$g_{cn1} + g_{cn2}$	Flickery	11	+80	1.1	3.8	+80	1.3	199, 35
		7.3	+60	1.9	2.9	+60	1.6	
Bovine (cloned)	ND	64	+80	2.9	1.5	+80	2.2	5
Frog (native)	19, 12–15, 21	3.4			2.1			170, 101, 332
		4.0	+80	1.6	1.5	+80	1.4	
		3.0	-60	1.3	4.5	-60	1.3	
Chilean toad (native)	22	30						133
Toad (native)	25	3.4	0	1.5	1.5	0	1.5	255, 178
		31	+50	2.1	13	+50	2.0	
Salamander (native)	45, 50.5	20	-50	2.1	4	-50	2.1	371, 42, 193
					1.3	-60	2.1	
Catfish (native)	44	2–3			2–3			49, 111
		3.4		1.4	2.5		1.3	
Catfish (cloned)	55	42		1.4	40		1.3	111
Carp (native)	51	1.3	-40	1.07	0.9	-40	1.12	169
<i>Drosophila</i> (cloned)	36	406	+80	1.5	13	+80	1.5	22

$K_{1/2}$ , apparent dissociation constant for cyclic nucleotide;  $u_m$ , holding membrane potential used for determination of  $K_{1/2}$  and  $n$ ;  $n$ , Hill coefficient as defined by Hill equation:  $I = I_{\max}[\text{cN}]^n / (K_{1/2}^n + [\text{cN}]^n)$ , where  $I$  is current recorded in presence of a concentration of cyclic nucleotide ( $[\text{cN}]$ ) and  $I_{\max}$  is current recorded with a saturating concentration of cyclic nucleotide.

larization to induce a small tonic increase in the rate of firing of action potentials but would be unlikely to result in the characteristic strong phasic increase in action potential firing rate that odorants elicit in olfactory neurons (106, 108). Hence, the major effect of the activation of  $g_{cn}$  is to increase  $[\text{Ca}^{2+}]_i$ , which then acts as a third messenger.

### C. Calcium-Activated Chloride Current

Kleene and Gesteland (165) first described a ciliary  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  conductance (Fig. 5). They postulated that the increase in  $[\text{Ca}^{2+}]_i$  resulting from the opening of cAMP-gated channels activates ciliary  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels, thereby amplifying the generator current (Fig. 3), a suggestion that has been confirmed by experiments from various laboratories (89, 160, 167, 184, 214, 361). These  $\text{Cl}^-$  channels display a steep dependence on  $[\text{Ca}^{2+}]_i$  (Hill coefficient of 2) with a  $K_{1/2}$  of 4.8  $\mu\text{M}$  (165). Calcium concentrations in this range can be reached easily because of the small volume of olfactory cilia (106, 284) and the restricted diffusion for  $\text{Ca}^{2+}$  in the cytosol (144, 259). In a ciliary compartment of 1 fl (0.2  $\mu\text{m}$  in diameter and  $\sim 30 \mu\text{m}$  in length), 1 pA  $\text{Ca}^{2+}$  influx corresponds to  $\sim 10^4 \text{Ca}^{2+}/10 \text{ms}$ . Adding this number of  $\text{Ca}^{2+}$  to a volume of 1 fl corresponds to an increase of total  $\text{Ca}^{2+}$  concentration of 100  $\mu\text{M}$ . If 99% of the  $\text{Ca}^{2+}$  entering the cilium is buf-

fered, as in other systems (118, 259), the increase of free  $[\text{Ca}^{2+}]_i$  would be 1  $\mu\text{M}/10 \text{ms}$ . This does not, of course, exclude much higher concentrations on a shorter time scale.

Calcium-activated  $\text{Cl}^-$  current resulting from this increase in  $[\text{Ca}^{2+}]_i$  is depolarizing because the reversal potential for  $\text{Cl}^-$   $u_{\text{Cl}}$  is considerably more positive than the resting potential in these neurons. Kurahashi and Yau (184) measured  $\text{Cl}^-$  inward currents using voltage clamp at a holding potential of -50 mV, indicating that  $u_{\text{Cl}} > -50 \text{mV}$  (Fig. 6). Dubin and Dionne (76) obtained a  $u_{\text{Cl}}$  value of -45 mV and Zhainazarov and Ache (361) determined a  $u_{\text{Cl}}$  value of -2.3 mV using the perforated patch technique (125) and the  $\text{Cl}^-$ -impermeable pore gramicidin. As a result, the opening of the  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels results not only in amplification of the odor-induced current, but also in enhanced stability of the transduction current in a wide variety of extracellular ionic environments (167, 184).

Presumably,  $u_{\text{Cl}}$  is not constant during activation of the  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels because of the small volume of the cilia: intracellular  $\text{Cl}^-$  concentration = 100 mM means  $\sim 60 \times 10^6 \text{Cl}^-/\text{cilium}$  ( $V_{\text{cilium}} = 1 \text{fl}$ ). On the other hand,  $60 \times 10^6$  is the number of ions that cross the membrane if a current of 10 pA over 1 s is assumed. A depletion of  $\text{Cl}^-$  within the cilia and a corresponding shift of  $u_{\text{Cl}}$  to more negative values can be expected during activation. This Goldman-Hodgkin-Katz-like mechanism would be a

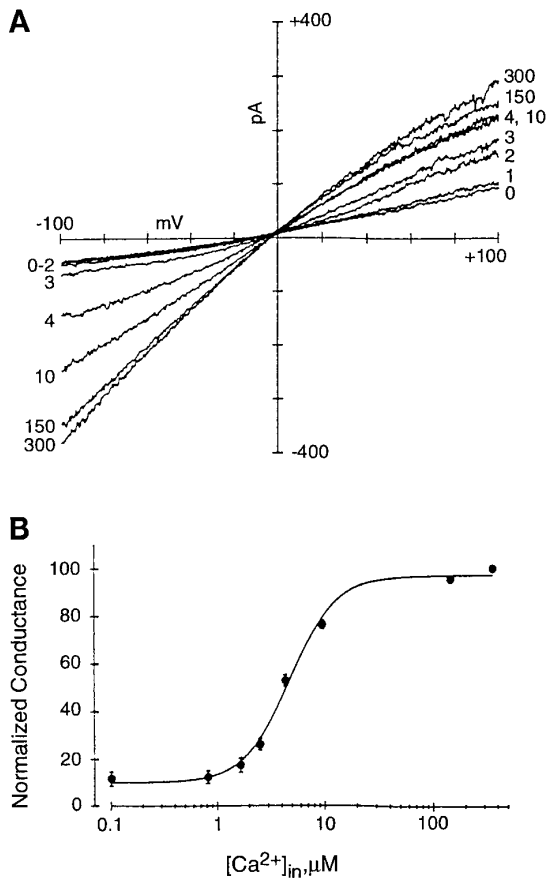


FIG. 5. Effect of cytoplasmic  $\text{Ca}^{2+}$  on ciliary membrane conductance of olfactory receptor neurons of northern grass frog, *Rana pipiens*. *A*: current-voltage relationship of membrane of 1 cilium was measured in each of 8 baths. Each bath consisted of a standard pseudointracellular solution plus free  $\text{Ca}^{2+}$  from 0 to 300  $\mu\text{M}$  as indicated. Recording pipette contained standard extracellular solution. *B*: slope of current-voltage plot, measured in linear range between  $-50$  and  $0$  mV, is plotted against concentration of free  $\text{Ca}^{2+}$  in bath. Each point is mean of determinations in 7 cilia. Conductance in 300  $\mu\text{M}$   $\text{Ca}^{2+}$  was arbitrarily defined as 100, and other values were normalized to this. Actual conductance in 300  $\mu\text{M}$   $\text{Ca}^{2+}$  was  $4.1 \pm 0.8$  nS ( $n = 7$ ; range, 1.8–8.3 nS). No controls have been subtracted. Curve is best-fitting Hill equation, which has a  $K_{1/2}$  of 4.8  $\mu\text{M}$  and a Hill coefficient of 2.0. [Modified from Kleene and Gesteland (165).]

negative feedback:  $I_{\text{Cl}} = g_{\text{Cl}(\text{Ca})}(u - u_{\text{Cl}})$ , where  $I_{\text{Cl}}$  is  $\text{Cl}^-$  current, would decrease because of a shift of  $u_{\text{Cl}}$  based on  $I_{\text{Cl}}$  itself. Future studies are necessary to better understand the  $\text{Cl}^-$  concentrations in ORNs and the mucus (see Table 4) as well as the mechanism(s) responsible for  $\text{Cl}^-$  ion homeostasis in ORNs.

Although the exact mechanisms of how bicarbonate permeation (31, 187),  $\text{Cl}^-$  exchangers (289), and pumps affect  $u_{\text{Cl}}$  remain to be elucidated, it appears that the major component of the cAMP-stimulated transduction current in ORNs is a  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  current. In retrospect, the initial failure of many laboratories to record odorant-induced currents in isolated receptor neurons may have resulted from choosing a physiological  $[\text{Ca}^{2+}]_o$

and setting a low  $u_{\text{Cl}}$ , whereas successful recordings were made using unphysiologically low  $[\text{Ca}^{2+}]_o$  and an unusual, but physiologically correct,  $u_{\text{Cl}}$ . However, under the latter conditions, the cation current through  $g_{\text{cn}}$  contributes significantly to the overall odor-gated current and was therefore initially assumed to be identical to it.

## D. Summary

The cAMP-mediated olfactory transduction cascade (Fig. 3) consists of seven TM receptors that couple to G proteins ( $G_{\text{olf}}$ ). An adenylyl cyclase (type III) is stimulated, and the resulting increase in cAMP directly activates cyclic nucleotide-gated channels ( $g_{\text{cn}}$ ). These channels are blocked by both  $[\text{Ca}^{2+}]_o$  and  $[\text{Ca}^{2+}]_i$ . Under physiological conditions, the  $g_{\text{cn}}$  channels are highly permeable for  $\text{Ca}^{2+}$ , which enters the cell and activates a  $\text{Ca}^{2+}$ -dependent  $\text{Cl}^-$  conductance ( $K_{1/2} = 4.8$  mM), the current through which is depolarizing because  $u_{\text{Cl}}$  is considerable less negative than the resting potential.

## VI. INOSITOL TRISPHOSPHATE-MEDIATED SIGNALING IN OLFACTORY CELLS

### A. $\text{InsP}_3$ Formation and Receptors

The involvement of a second messenger other than cAMP in mediating olfactory transduction was first suggested by biochemical studies with isolated olfactory cilia showing that certain odorants fail to elicit substantial increases in cAMP formation (49, 326). For some of these odorants, the weak responsiveness in biochemical experiments might be explained by the possibility that the odor-

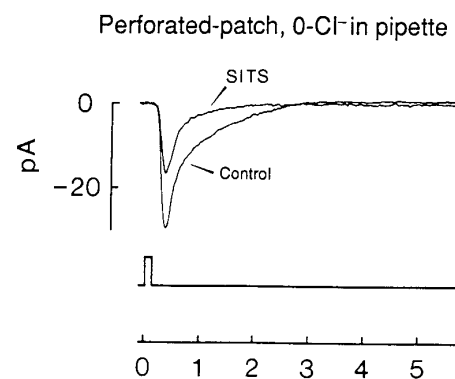


FIG. 6. Perforated-patch recording from a dissociated newt (*C. pyrrhogaster*) olfactory receptor neuron. Patch pipette contained no  $\text{Cl}^-$  to avoid loading cell with  $\text{Cl}^-$ . Holding potential was  $-50$  mV. Chloride channel blocker 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS) blocks a substantial part of odor-induced current (top trace). Time course of odorant stimulation is shown below current trace. A mixture of *n*-amylacetate, isoamylacetate, and limonene was used as stimulus. [From Kurahashi and Yau (184).]

ants elicit increases in cAMP in a small fraction of olfactory neurons as indicated by the small magnitude of their EOG responses (216). However, odorants like lylal, isovaleric acid, and isobutyric acid in frog (216), trimethylamine, isovaleric acid, and lilial in mouse (50) and L-cysteine in catfish (49) elicit relatively strong EOG responses but fail to elicit a substantial increase in cAMP formation (37, 49, 326).

Huque and Bruch (127) first determined that certain amino acids known to be potent odorants in catfish (53, 334) elicit increases in the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP<sub>2</sub>) to diacylglycerol (DAG) and InsP<sub>3</sub> in a GTP-dependent manner. These experiments, performed with isolated catfish olfactory cilia, showed that the elevation in InsP<sub>3</sub> formation was absolutely dependent on GTP, implicating a G protein in this response. These and other studies with conventional biochemical methods using membrane preparations (208, 210) or olfactory neuronal cultures (297) have been confirmed and extended in experiments in the subsecond time range utilizing stopped flow methods that demonstrated rapid transient stimulation of cAMP and/or InsP<sub>3</sub> formation in a variety of species (27, 28, 37, 38, 287, 297, 308). In addition, stopped flow measurements showed that stimulation of both second messenger pathways by odorants was dependent on guanine nucleotides (28, 208, 210, 287) and that the cAMP and InsP<sub>3</sub> pathways were differentially sensitive to bacterial toxins (28), implicating different G proteins in mediating the cAMP and InsP<sub>3</sub> responses. Finally, Raming et al. (281) have shown in a preliminary study that Sf9 insect cells transfected with a putative olfactory receptor responded to odorants with a dose-dependent increase in InsP<sub>3</sub> formation.

The identity of the G protein(s) that couples odorant receptors to InsP<sub>3</sub> formation is not firmly established. In other cell systems, phospholipase C-catalyzed InsP<sub>3</sub> formation is activated by G protein  $\alpha$ -subunits of the G<sub>q</sub> and G<sub>o</sub> types, by the  $\beta, \gamma$ -subunits released after the interaction of G protein heterotrimer with ligand-bound receptor, or by tyrosine kinase receptors (79). In stopped flow studies, Breer and co-workers (28) found that the odorant-stimulated InsP<sub>3</sub> response was inhibited by pertussis toxin, suggesting that the G protein mediating InsP<sub>3</sub> formation in the olfactory system is pertussis toxin sensitive (28). A number of laboratories have localized immunoreactivity to G<sub>q</sub>, a G protein known to be linked to InsP<sub>3</sub> formation in other cell systems, to the olfactory cilia (1, 71, 237). In addition, in cultured lobster olfactory neurons, antibodies raised against G<sub>q</sub> and G<sub>o</sub> inhibit InsP<sub>3</sub>-mediated whole cell responses (82). This indicates that G<sub>q</sub> and G<sub>o</sub> could be G proteins linking olfactory receptors to InsP<sub>3</sub> formation. However, in contradiction to the studies of Breer and co-workers (28), G<sub>q</sub> is not a pertussis toxin-sensitive G protein. Therefore, it remains unclear whether G<sub>q</sub> or a pertussis toxin-sensitive G<sub>q</sub>-like G protein is involved in olfactory transduction.

Inositol trisphosphate receptors have been characterized biochemically in isolated olfactory cilia. Isolated olfactory cilia from catfish and rat have been shown to be enriched with an InsP<sub>3</sub>-binding protein that displayed stereospecificity and affinity consistent with olfactory InsP<sub>3</sub>-gated conductances studied in bilayers (see sect. vIB2) but different from that of the InsP<sub>3</sub> receptor found in the endoplasmic reticulum (ER) of CNS neurons (141, 294). Antibodies raised against rat brain InsP<sub>3</sub> receptors labeled the olfactory cilia layer (67).

## B. Inositol Polyphosphate-Regulated Conductance(s)

### 1. *InsP<sub>3</sub>-regulated Ca<sup>2+</sup> conductance*

In *Xenopus* olfactory neurons, dialysis of InsP<sub>3</sub> into the cytoplasm resulted in opening of an apically localized Ca<sup>2+</sup>-permeable conductance ( $g_{Ca,IP_3}$ ) (Fig. 7). This conductance appears to have a stereospecificity different from the InsP<sub>3</sub>-gated nonspecific cation conductance ( $g_{cat,IP_3}$ ) discussed in section vIB2, since  $g_{Ca,IP_3}$  is activated by addition of Ins(1,4,5)P<sub>3</sub> but not by stimulation with Ins(2,4,5)P<sub>3</sub> (311). More importantly, a necessary condition for the activation of  $g_{Ca,IP_3}$  by InsP<sub>3</sub> is that  $[Ca^{2+}]_i$  is higher than  $\sim 160$  nM (311). The identity of the ionic channel(s) underlying  $g_{Ca,IP_3}$  has not been determined. Interestingly, a similar InsP<sub>3</sub>-regulated Ca<sup>2+</sup>-selective conductance has been identified in insect ORNs where it is believed to contribute to the early phase of the response to the pheromone bombykal (328).

### 2. *Nonspecific cation conductances*

Electrophysiological whole cell experiments with catfish (*Ictalurus punctatus*) ORNs and with isolated catfish olfactory cilia fused onto an artificial lipid bilayer first suggested that InsP<sub>3</sub> regulates a nonspecific cation conductance  $g_{cat,IP_3}$  in ORNs (290). The InsP<sub>3</sub>-gated channel from catfish had a conductance of 79 pS. It was inhibited by ruthenium red (5  $\mu$ M) but not by nimodipine (5  $\mu$ M). The channel was a nonspecific cation channel with a slightly higher permeability for divalent cations than monovalent cations ( $P_{Ba} = 7P_K$ ). The authors acknowledged the possibility that the InsP<sub>3</sub>-gated channels incorporated into the artificial lipid bilayers could be introduced by ER contamination of the cilia membrane preparation. However, they argued that this was presumably not the case because a preparation of ER from deciliated olfactory epithelium did not yield any channel activity, and because the frequency for finding an InsP<sub>3</sub> channel was comparable to the frequency for finding cAMP-gated channels that are believed to be located in the cilia.

Since then, several studies have been published con-

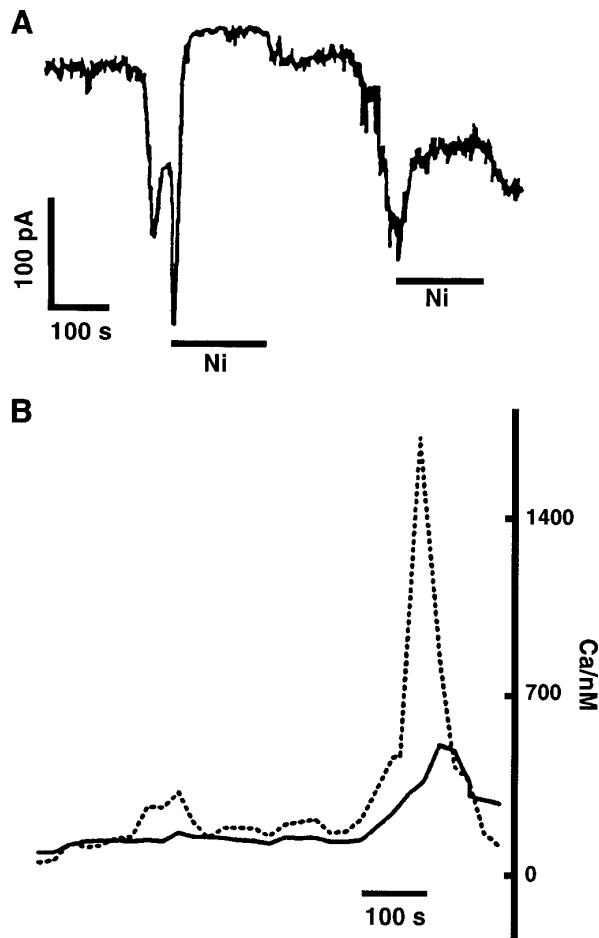


FIG. 7. Simultaneous current and charge-coupled device intracellular  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) recording in an olfactory receptor neuron of clawed frog, *Xenopus laevis*, upon dialysis of  $100 \mu\text{M}$  inositol trisphosphate ( $\text{InsP}_3$ ) through patch pipette. *A*: current recording at a holding potential of  $-80 \text{ mV}$ . First response was completely blocked by  $\text{Ni}^{2+}$  (Ni), whereas second response was only partly blocked by  $\text{Ni}^{2+}$ . *B*: simultaneous with current measurement shown in *A*,  $[\text{Ca}^{2+}]_i$  was imaged and evaluated in dendritic knob (dotted line) and soma (continuous line). Time axes in *A* and *B* are identical. [Modified from Schild et al. (311).]

firming the presence of  $\text{InsP}_3$ -gated nonspecific cation channels in the plasma membrane of ORNs from a variety of species. In spiny lobster *Panulirus argus*, Ache and co-workers demonstrated the presence of  $\text{InsP}_3$ -gated nonspecific cation channels in plasma membrane patches from cultured lobster ORNs (80) and in patches from the outer dendrite of lobster ORN (116). Because in the lobster the outer dendritic segment is believed to be the site where olfactory transduction takes place (morphologically equivalent to the cilia of vertebrate olfactory neurons), these observations support the role for these channels in olfactory transduction. Two channels differing in conductance and kinetics were described in lobster ORN. One channel has a conductance of  $27 \text{ pS}$ , and its open probability is strongly voltage dependent, being nearly

zero at  $-70 \text{ mV}$ , and rising to  $0.78$  at  $+70 \text{ mV}$ . The other channel has a larger conductance ( $64 \text{ pS}$ ), and its open probability is less voltage dependent. Both channels are nonspecific cation channels with a larger permeability for  $\text{K}^+$  than for  $\text{Na}^+$  ( $P_{\text{K}}/P_{\text{Na}}$  ranges from 4 to 6, calculated from reversal potentials reported in Ref. 116 under the assumption that anions do not permeate through the channel). The permeability of the lobster cation channels for divalent cations has not been reported.

Isolated olfactory cilia from rat (*Rattus rattus*) incorporated into artificial lipid bilayers also display an  $\text{InsP}_3$ -gated nonspecific cation conductance ( $g_{\text{cat,IP}_3}$ ) (294). Activation by inositol polyphosphates is stereospecific, and the potency of different inositol phosphates correlates with the binding affinities measured biochemically in isolated olfactory cilia. Inositol 2,4,5-trisphosphate was the most potent analog, activating the channel at nanomolar concentrations;  $\text{Ins}(1,4,5)\text{P}_3$  activated at micromolar concentrations, whereas inositol 1,4-bisphosphate did not activate the channel ( $5\text{--}15 \mu\text{M}$ ). Two types of channels could be discerned at the single-channel level (123). One had a conductance of  $37 \text{ pS}$  and displayed a characteristic shift in open amplitude as a function of open time. The other channel had a larger conductance ( $103\text{--}184 \text{ pS}$ ). Addition of  $\text{InsP}_3$  ( $1 \mu\text{M}$ ) to membrane patches excised from the soma of frog and mouse ORNs elicits current fluctuations in otherwise silent bilayers (333). The chord conductance for the  $\text{InsP}_3$ -gated channels from frog recorded at a holding potential of  $-86 \text{ mV}$  was  $20 \text{ pS}$ .

The presence of  $\text{InsP}_3$ -regulated conductances has also been assessed by dialysis of  $\text{InsP}_3$  into the cytosol of ORNs under whole cell patch clamp. Under these conditions, however, it cannot be concluded that the effect of  $\text{InsP}_3$  is direct. Dialysis of  $\text{InsP}_3$  into ORNs from several species ranging from lobster to rats elicits opening of a  $g_{\text{cat,IP}_3}$  (80, 145, 245, 264, 290, 311, 327, 333). The effect of inositol phosphates on this conductance is stereospecific (264, 311). The  $g_{\text{cat,IP}_3}$  is permeable to monovalent cations ( $\text{Na}^+$ ,  $\text{K}^+$ , and choline) as well as to divalent cations including  $\text{Ca}^{2+}$  (145, 311). The  $g_{\text{cat,IP}_3}$  is inhibited by internally applied ruthenium red in rat (264) and by externally applied ruthenium red in fish (245, 290) and lobster (80). The conductance is also blocked by cadmium (264) and nickel (311). The shape of the  $I\text{-}V$  relationship for  $g_{\text{cat,IP}_3}$  is either linear or outwardly rectifying. Simultaneous recording of current and  $\text{Ca}^{2+}$  concentration shows that  $g_{\text{cat,IP}_3}$  is capable of eliciting substantial changes in  $[\text{Ca}^{2+}]_i$  (145, 311). The increase in  $[\text{Ca}^{2+}]_i$  elicited by dialysis of  $\text{InsP}_3$  from the patch pipette is dependent on the presence of extracellular  $\text{Ca}^{2+}$  (145).

Simultaneous measurements of  $[\text{Ca}^{2+}]_i$  and whole cell current in *Xenopus* ORNs that have kept their characteristic bipolar morphology after isolation suggest an apical localization for the  $g_{\text{cat,IP}_3}$  (311) (Fig. 7). The apical localization of  $g_{\text{cat,IP}_3}$  has been confirmed in rat by patch-clamp

experiments in which the entire dendrite including the olfactory knob and cilia are excised (121) and in excised patches of outer dendrite plasma membrane from lobster ORNs (116).

### 3. Inositol tetrakisphosphate-gated channel

A novel inositol tetrakisphosphate (InsP<sub>4</sub>)-gated cation channel with a conductance of 194 pS has been described in lobster olfactory neurons (81). This channel was localized to the outer dendritic membrane in lobster ORNs (116). With the assumption of the presence of a InsP<sub>3</sub> 3-kinase, the production of InsP<sub>3</sub> would be followed by substantial increases in Ins(1,3,4,5)P<sub>4</sub>, and the InsP<sub>4</sub>-gated channel could participate in olfactory transduction by enhancing and/or modifying the response that would be obtained with InsP<sub>3</sub> alone. The InsP<sub>4</sub>-gated channel has been found to be in close spatial proximity and to interact with the InsP<sub>3</sub>-gated conductance in lobster ORNs (81).

### 4. Negative evidence

In contrast to the results discussed above, there are a substantial number of studies that report little or no effect of InsP<sub>3</sub> on plasma membrane conductance in ORNs. A number of laboratories have reported the lack of an effect of membrane conductance on dialysis of InsP<sub>3</sub> into the cytosol of olfactory neurons under whole cell patch clamp (86, 213, 257), and two groups have reported failure of InsP<sub>3</sub> to elicit a change in membrane conductance in excised olfactory cilia under voltage clamp (166, 256). Additionally, and in contrast to studies in *X. laevis* (311) and frog (145), in newt ORNs InsP<sub>3</sub> failed to elicit an increase in apical [Ca<sup>2+</sup>]<sub>i</sub> (257). These newt ORNs, which had a very short dendrite, responded within 15 s to dialysis of cAMP into the cytoplasm with an apical increase in [Ca<sup>2+</sup>]<sub>i</sub>, whereas [Ca<sup>2+</sup>]<sub>i</sub> increased over 1 min in the soma when the cells were dialyzed with Ins(1,4,5)P<sub>3</sub>. On the basis of these data, the authors proposed that cAMP directly mediates transduction while InsP<sub>3</sub> mediates adaptation through the control of [Ca<sup>2+</sup>]<sub>i</sub> by release of Ca<sup>2+</sup> from internal stores. Finally, on the basis of general anosmia of transgenic mice deficient for the olfactory cAMP-gated channel, Brunet et al. (50) concluded that cAMP is the only second messenger mediating olfactory transduction.

The reason for the discrepancy between these negative results and the results reviewed in the preceding sections is presently not clear. The interpretation of the currently available data by different investigators ranges from an exclusive role of cAMP in olfactory transduction to a mediatory role for cAMP and a modulatory role for InsP<sub>3</sub>, to dual mediation of olfactory transduction by the two second messengers. A definitive answer of this question

awaits further research, and whatever the answer will be, it will explain the observations reviewed above.

The following have been offered as possible explanations for the apparent discrepancies presented above: 1) species differences; 2) the use in some studies of very low intracellular [Ca<sup>2+</sup>]<sub>i</sub>, which has been shown to prevent the InsP<sub>3</sub> response in *Xenopus* ORNs (311); and 3) the presence of a high concentration of ATP in the pipette which antagonizes binding of InsP<sub>3</sub> to InsP<sub>3</sub> receptors (311). 4) In some species, InsP<sub>3</sub> may elicit small current responses that could be overlooked in electrophysiological experiments. If in some species the InsP<sub>3</sub> response is mediated mainly by InsP<sub>3</sub>-regulated Ca<sup>2+</sup> channels (311), then only a very small current would be required to elicit large changes in [Ca<sup>2+</sup>]<sub>i</sub>. 5) Some of the components of the InsP<sub>3</sub> pathway may be damaged during cell isolation. In particular, *X. laevis* ORNs whose dendrite has shortened during isolation do not respond to dialysis of InsP<sub>3</sub> from the pipette (F. Lischka and D. Schild, unpublished observations). 6) In reference to the work of Brunet et al. (50) with a cAMP-gated channel-deficient mouse strain, controls were not presented to address possible pleiotropic effects of the knockout on the InsP<sub>3</sub> pathway. 7) In addition, this study was carried out using EOG recordings as the only measure for the responsiveness of ORNs. However, EOG recordings are an indirect measurement of ORN activity that depend on a number of parameters unrelated to the magnitude of the generator current, for instance, the exact position of the EOG electrode, the number of cells under the macroelectrode, and the resistance of the paracellular junction. Hence, measurements with EOGs, although suggestive, do not conclusively show that the generator current is affected. Future studies must address these points.

### C. Electrophysiological Studies of InsP<sub>3</sub>-Mediated Odor Responses

The most direct evidence for odor responses mediated by opening of InsP<sub>3</sub>-gated channels is found in invertebrates (spiny lobster *P. argus* ORNs). Fadool and Ache (80) showed that an inward current induced by a mixture of odorants (tetramine) in cultured lobster ORNs is enhanced fourfold by addition of an antibody raised against the COOH terminus of the mammalian InsP<sub>3</sub> receptor. The same antibody increased the open probability of InsP<sub>3</sub>-gated ion channels recorded in excised patches or lobster ORN plasma membrane by a factor of 4. The *I-V* relationship and pharmacology of the odorant-induced inward current in lobster are currently unknown.

In vertebrates, the only published report of the ionic basis of responses to odorants known to elicit InsP<sub>3</sub> formation in biochemical experiments in the same species is from catfish ORNs (130, 246). Extracellular recordings from single units in the channel catfish *I. punctatus* indicate that amino

acids elicit both excitatory and suppressive responses in individual olfactory neurons (142). Biochemical experiments with isolated olfactory cilia from catfish indicate that amino acid odorants stimulate formation of  $\text{InsP}_3$  (48, 287), although formation of cAMP was also stimulated at high concentration and after long exposure to the odorant (49). A single stimulus could be excitatory in one cell by eliciting an increase in the frequency of action potential firing and cause inhibition of action potential firing in another cell, and a single cell could be excited by one amino acid and inhibited by another. These experiments suggest at least two pathways for olfactory signal transduction in catfish. In experiments with freshly isolated catfish ORN under whole cell patch clamp, some cells responded to amino acids with inward currents that reversed near 0 mV, consistent with either nonspecific cation channels or a  $\text{Cl}^-$  conductance (130, 246). In some of these cells, the current was presumably carried by a cAMP-gated nonspecific cation current as evidenced by the marked outward rectification in the presence of extracellular divalent cations, and the sensitivity of the response to pretreatment with forskolin (246). However, in other cells, the response reversed at positive potentials (23 mV), and the response was augmented, and the reversal potential became more positive upon addition of 1 mM extracellular  $\text{Ca}^{2+}$  (246). This later response cannot be mediated by nonspecific cation or  $\text{Cl}^-$  conductances alone and is consistent with mediation by a  $\text{Ca}^{2+}$ - or  $\text{Na}^+$ -permeable or an inwardly rectifying cation conductance. It is at present unclear whether this odor-activated conductance is regulated by  $\text{InsP}_3$  or another second messenger.

Two groups have reported responses to odorants known to stimulate  $\text{InsP}_3$  formation in biochemical experiments with rat olfactory cilia in frog ORNs. Working with *Rana ridibunda* ORNs, Kashiwayanagi (145) found that a mixture of lilyl, lylal, and ethyl vanillin elicited a substantial (-41 pA on the average) increase in inward current at a holding potential of -70 mV with a parallel increase in  $[\text{Ca}^{2+}]_i$ . These odorants elicited a response even in ORNs exposed to 1 mM cAMP by dialysis through the patch pipette. In contrast, in Chilean toad (*Caudiverbera caudiverbera*), a mixture of isovaleric acid, pyrazine, and triethylamine elicited a small (5 pA) inward current in the presence of 10 mM extracellular  $\text{BaCl}_2$ . In the presence of amphibian Ringer solution in the bath, these investigators were unable to detect an odorant-induced inward current but detected an odorant-induced increase in  $[\text{Ca}^{2+}]_i$  in the dendritic knob (16). The increase in  $[\text{Ca}^{2+}]_i$  elicited opening of  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels in this preparation (discussed in sect. VII). The nature of the second messenger mediating the odorant response in *C. caudiverbera* is unknown.

#### D. Summary

The  $\text{InsP}_3$ -mediated olfactory transduction pathway is much less analyzed than the cAMP-mediated pathway,

and many steps of the signaling cascade are as yet not understood. Some odorants that elicit EOG responses fail to stimulate cAMP formation. Instead, some of these odorants lead to an increase of the concentration of the second messenger  $\text{InsP}_3$ . In ORNs dialyzed with  $\text{InsP}_3$  through the patch pipette, a clear increase in  $[\text{Ca}^{2+}]_i$  was observed in the dendritic ending and, simultaneously, a nonspecific cation current as well as a  $\text{Ca}^{2+}$  current were measured. The tentative scheme of this pathway would thus assume receptors that couple to G proteins and a phospholipase C. Upon activation of this cascade,  $\text{InsP}_3$  is formed and leads to an increase in  $[\text{Ca}^{2+}]_i$ , which could activate either  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  channels or  $\text{Ca}^{2+}$ -dependent nonspecific cation channels.

### VII. CALCIUM AS A THIRD MESSENGER IN OLFACTORY TRANSDUCTION

As discussed above, odorants induce increases in cAMP and/or  $\text{InsP}_3$ , which activate  $\text{Ca}^{2+}$ -permeable conductances and increase  $\text{Ca}^{2+}$  concentration. Odorants thus cause an increase in  $[\text{Ca}^{2+}]_i$  in olfactory neurons (16, 145, 290–292, 302, 337) (see also sect. IV E). Even after prolonged stimulation, the odor-induced increases in  $[\text{Ca}^{2+}]_i$  are spatially inhomogeneous, with the largest changes occurring in the apical portion of the cells (16, 290, 302, 337). Studies of the effects of the cAMP and/or  $\text{InsP}_3$ -induced elevation in  $[\text{Ca}^{2+}]_i$  have made it increasingly clear that  $\text{Ca}^{2+}$  plays the role of a third messenger by eliciting opening of  $\text{Ca}^{2+}$ -regulated conductances, thereby greatly amplifying or even changing the polarity of the generator potential (Fig. 8).

Three ciliary  $\text{Ca}^{2+}$ -activated conductances thought to be regulated by odorant-induced increases in  $[\text{Ca}^{2+}]_i$  have been described in ORNs: a  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  conductance, a  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  conductance, and a  $\text{Ca}^{2+}$ -activated cation conductance ( $g_{\text{can}}$ ). In the case of the cAMP-mediated transduction pathway,  $\text{Ca}^{2+}$  concentration activates a  $\text{Cl}^-$  current (160, 165, 184, 214, 361), which underlies the receptor potential (see sect. V C).

With regard to the action of an  $\text{InsP}_3$ -mediated increase of  $[\text{Ca}^{2+}]_i$ , there are divergent reports. Evidence for three possibilities has been accumulated: 1) activation of a  $\text{Ca}^{2+}$ -dependent nonspecific cation conductance,  $g_{\text{can}}$ ; 2) activation of a  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  conductance,  $g_{\text{K}(\text{Ca})}$ ; and 3) activation of both  $g_{\text{can}}$  and  $g_{\text{K}(\text{Ca})}$ .

1) The activation of a  $\text{Ca}^{2+}$ -dependent nonspecific cation conductance  $g_{\text{can}}$  in vertebrate ORNs has first been demonstrated in *Xenopus* olfactory neurons (307). This conductance is situated in the transduction compartments of *Xenopus* ORNs as shown by simultaneous patch-clamp recordings and  $[\text{Ca}^{2+}]_i$  imaging (310). Its ionic properties as well as the modulation by  $[\text{Ca}^{2+}]_i$  and  $[\text{Ca}^{2+}]_o$  differ markedly from the corresponding properties of the cAMP-

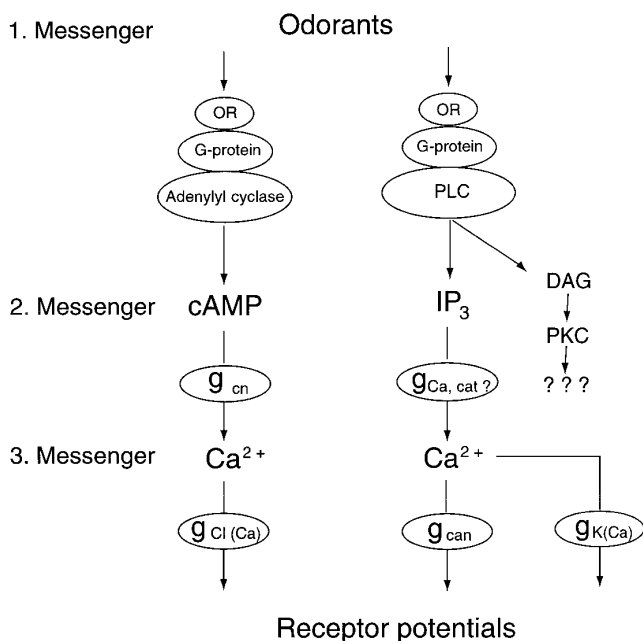


FIG. 8. Schematic diagram showing parallels between cAMP-mediated pathway and presumed  $\text{InsP}_3/\text{Ca}^{2+}$ -mediated pathway. In either case, odorants act via olfactory receptors (OR), G proteins (G), and an enzyme that forms a second messenger, cAMP or  $\text{InsP}_3$ . Second messenger activates a  $\text{Ca}^{2+}$ -permeable conductance. Resulting increase in  $[\text{Ca}^{2+}]_i$  then activates a second conductance, e.g., a  $\text{Cl}^-$ , a cation, or a  $\text{K}^+$  conductance, thereby leading to generation of receptor potential. PLC, phospholipase C; DAG, diacylglycerol; PKC, protein kinase C;  $g_{\text{cn}}$ , nucleotide-activated conductance;  $g_{\text{Ca, cat}}$ ,  $\text{Ca}^{2+}$  conductance or  $\text{Ca}^{2+}$ -permeable cation conductance;  $g_{\text{Cl(Ca)}}$ ,  $\text{Ca}^{2+}$ -dependent  $\text{Cl}^-$  conductance;  $g_{\text{can}}$ ,  $\text{Ca}^{2+}$ -dependent cation conductance;  $g_{\text{K(Ca)}}$ ,  $\text{Ca}^{2+}$ -dependent  $\text{K}^+$  conductance.

gated cation conductance  $g_{\text{cn}}$  (Table 3) so that  $g_{\text{can}}$  and  $g_{\text{cn}}$  appear to be different conductances. Its properties further suggest that  $g_{\text{can}}$  is identical to the nonspecific cation conductance stimulated by dialysis of  $\text{InsP}_3$  into the cytoplasm of *Xenopus* olfactory neurons ( $g_{\text{cat,IP}_3}$ ) (see Table 3) (311), although the molecular identity of  $g_{\text{can}}$  has not been demonstrated directly. It remains to be estab-

lished whether the action of  $\text{InsP}_3$  on  $g_{\text{can}}$  is direct. In any case, the resulting current is depolarizing at resting membrane potential.

2) In Chilean toad *C. caudiverbera*, there is evidence implicating odorants known to stimulate  $\text{InsP}_3$  formation in isolated olfactory cilia from rat in an inhibitory (hyperpolarizing) response (248, 249). In ORNs from this species, odorants such as isovaleric acid, pyrazine, and triethylamine elicit a hyperpolarization that is mediated by opening of charybdotoxin-sensitive  $\text{K}^+$  channels, presumably  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels of the BK type (133, 248). Morales and co-workers (16) have shown that these odorants elicit an apically localized increase in  $[\text{Ca}^{2+}]_i$  due to influx of  $\text{Ca}^{2+}$  from the outside. The  $\text{Ca}^{2+}$  conductance is sensitive to nifedipine, an inhibitor of L-type  $\text{Ca}^{2+}$  channels as well as of cAMP-gated channels (370). The identity of this odorant-activated  $\text{Ca}^{2+}$  conductance in toad is unknown, and it remains to be determined whether its activation is mediated by second messengers (cAMP or  $\text{InsP}_3$ ).

The net result of the opening of the  $\text{K}^+$  channel would be to turn a response that would otherwise be depolarizing into a hyperpolarizing (suppressing) response. This is consistent with the fact that odorants that stimulate this  $\text{K}^+$  conductance elicit suppression of the basal rate of firing of action potentials in Chilean frogs. It has, however, to be kept in mind that mucosal  $\text{K}^+$  concentration can be much higher than the basolateral  $\text{K}^+$  concentration (see sect. IX), which would result in a less negative reversal potential for  $\text{K}^+$  and turn the activation of  $\text{K}^+$  channels localized to the mucosal side into a depolarization.

3) In catfish olfactory receptor cells, a charybdotoxin-sensitive  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  conductance was activated by dialysis of  $\text{InsP}_3$  into the cytoplasm, but this current developed more slowly than the  $\text{InsP}_3$ -gated nonspecific cation conductance (245). Consequently, it was postulated that in catfish this current participated in repolarization of the plasma membrane after prolonged stimulation. Similarly, in rat olfactory neurons, dialysis of  $\text{InsP}_3$  into the cytosol elicited opening of a  $\text{Ca}^{2+}$ -activated  $\text{K}^+$

TABLE 3. Properties of four cation conductances proposed to be involved in transduction in olfactory receptor neurons

	$g_{\text{cn}}$	$g_{\text{can}}$	$g_{\text{cat,InsP}_3}$	$g_{\text{Ca,InsP}_3}$
$\text{K}^+$ and $\text{Na}^+$ permeable	Yes	Yes	Yes	No
$\text{Ca}^{2+}$ permeable	Yes, but permeation block	Yes	Yes	Yes
$[\text{Mg}^{2+}]_o$	Block at $u < -70$ mV	No effect	??	??
$[\text{Ca}^{2+}]_i$	Block via calmodulin	Activates above 450 nM	Prevents opening below 280 nM	Prevents opening below 160 nM
Amiloride	Block	No block	No block	??
$\text{Ni}^{2+}$	Block	Partial block	Partial block	Block
Choline permeability	Very low	Permeates	Permeates	Very low
Reference no.	22, 56, 72, 101, 102, 163, 170, 173, 175, 176, 179, 206, 223, 255, 370	307, 310	80, 145, 245, 264, 290, 294, 311	311

$[\text{Mg}^{2+}]_o$ , extracellular  $\text{Mg}^{2+}$  concentration;  $[\text{Ca}^{2+}]_i$ , intracellular  $\text{Ca}^{2+}$  concentration;  $g_{\text{cn}}$ , cyclic nucleotide-activated conductance;  $g_{\text{can}}$ ,  $\text{Ca}^{2+}$ -dependent cation conductance;  $g_{\text{cat,InsP}_3}$ , inositol trisphosphate ( $\text{InsP}_3$ )-dependent cation conductance;  $g_{\text{Ca,InsP}_3}$ ,  $\text{InsP}_3$ -dependent  $\text{Ca}^{2+}$  conductance.

conductance, but the overall current (the sum of the  $K^+$  and nonspecific cation component) reversed at  $-35$  mV, a potential more positive than the resting potential for these cells (264). Indeed, under whole cell current clamp, catfish and rat olfactory neurons responded to increased cytosolic  $InsP_3$  with membrane depolarization, not hyperpolarization (245, 264).

In summary,  $Ca^{2+}$  seems to play the role of a third messenger in olfactory neurons. In the cAMP-mediated transduction pathway, it is increased by  $Ca^{2+}$  entering the cell through cyclic nucleotide-gated channels, whereas in the  $InsP_3$ -mediated transduction pathway, it may either be released from  $Ca^{2+}$  stores or enter the cells through cation channels. There are four different actions of  $Ca^{2+}$  reported in ORNs: 1) it activates a  $Ca^{2+}$ -dependent  $Cl^-$  conductance, 2) it activates a  $Ca^{2+}$ -dependent nonspecific cation conductance, 3) it activates a  $Ca^{2+}$ -dependent  $K^+$  conductance, or 4) it activates, in the same cell, both a  $Ca^{2+}$ -dependent nonspecific cation conductance and a  $Ca^{2+}$ -dependent  $K^+$  conductance, the overall effect being a depolarization.

### VIII. GASEOUS MESSENGERS IN OLFACTORY NEURONS (CARBON MONOXIDE AND NITRIC OXIDE)

Nitric oxide and carbon monoxide (CO) are recognized as gaseous second messengers that can act as local neurotransmitters because of their ability to diffuse across cell membranes and stimulate the soluble form of guanylate cyclase (36, 103, 350). A role for gaseous second messengers in signal transduction in the peripheral olfactory system was first proposed by Breer and Shepherd (41) based on experiments with isolated rat olfactory cilia, demonstrating that odorants stimulate slow increases in cGMP formation that are abolished by addition of inhibitors of NO synthase, the enzyme that catalyzes the formation of NO from L-arginine (39). These authors speculated that the increase in cGMP could activate the cyclic nucleotide-gated channel, which is slightly more sensitive to cGMP than cAMP (Table 2). Subsequent experiments by Lischka and Schild (205) in *Xenopus* ORNs showed that NO induced an inward current at negative holding potentials with the ionic characteristics of the cAMP-gated conductance. The development of this current was dependent on the presence of GTP in the pipette, suggesting that the inward current induced by NO was mediated through an elevation in cGMP concentration. In addition, a direct stimulatory effect of NO on cyclic nucleotide-gated channels has been shown in tiger salamander olfactory neurons (42). Nitric oxide causes a chemical modification at a cysteine residue in the region of the cAMP-gated channel linking the S6 transmembrane domain to the ligand-binding domain. This chemical modification favors the open-

channel conformation. Nitric oxide has also been shown to elicit opening of the second subunit of the cAMP-gated channel ( $g_{cn2}$ ) (44). These observations suggest that odorant-stimulated NO formation plays a role in olfactory transduction. However, immunohistochemical studies show that mature olfactory neurons do not express the neuronal form of NO synthase (70, 174, 299, 362). Possible explanations for the lack of expression of neuronal NO synthase in mature ORN may be expression of another form of NO synthase in the olfactory epithelium (70), or a role for NO in signal transduction at early stages in the development of the olfactory epithelium, with a switch to CO in later stages (193).

In contrast, heme oxygenase II, the enzyme catalyzing the degradation of heme to biliverdin and CO, has been localized to ORNs in adult rats (350). In addition, primary cultures of embryonic rat olfactory neurons produce CO, and changes in the amount of CO produced as a function of time in culture parallel changes in cGMP levels in the embryonic ORNs (129). Furthermore, addition of CO to freshly isolated tiger salamander (*A. tigrinum*) ORNs elicits opening of a conductance with the characteristics of the cyclic nucleotide-gated conductance (193). As in the case of NO (205), the effect of CO on this conductance is absolutely dependent on the presence of GTP in the patch pipette (193), suggesting that the effect of CO is mediated by cGMP.

Because the biochemically measured odor-induced increases in cGMP concentration in isolated olfactory cilia take place slowly, Breer and Shepherd (41) proposed a role for gaseous second messengers in olfactory adaptation. Indeed, Leinders-Zufall et al. (194) have shown that prolonged exposure to the membrane-permeable cGMP analog 8-bromo-cGMP elicits a decrease in the sensitivity of the response to odors in tiger salamander ORNs. The decrease in odorant sensitivity can also be elicited by prolonged exposure of the cells to CO. Furthermore, the effect of CO is absolutely dependent on the presence of extracellular  $Ca^{2+}$ , suggesting that it is mediated by the changes in  $[Ca^{2+}]_i$  elicited by opening of the cyclic nucleotide-gated (CNG) channels. On the basis of these experiments and the effect of inhibitors of the CO/cGMP pathway on olfactory adaptation, Zufall and Leinders-Zufall (375) suggest that CO/cGMP are involved in long-term adaptation in ORNs. Alternatively,  $[Ca^{2+}]_i$  could be simply modulated by  $[Ca^{2+}]_o$ . Interestingly, what on the surface appears to be an opposite effect has been suggested for exposure of ORNs to the other gaseous second messenger NO. In *Xenopus* ORNs, exposure to NO prevented washout of odor-induced currents measured under whole cell patch clamp (205). The apparent discrepancy in the effects of cGMP and gaseous second messengers on odor responses in salamander and *Xenopus* ORNs may be due to differences in the experimental protocols. A crucial parameter, which was different in the experiments made in the tiger salamander (193) and in *Xenopus* (205), was the



GTP concentration. With 1 mM (salamander) or 3 mM (*Xenopus*) GTP, both NO released from sodium nitroprusside and CO activated the cyclic nucleotide-dependent conductance  $g_{cn}$  (193, 205). But are GTP concentrations between 1 and 3 mM physiological? Among the almost nonexistent reports on cytosolic GTP concentrations, Kleinecke et al. (168) determined the concentration of total GTP to be  $\sim 300 \mu\text{M}$  in hepatocytes. In yeast, the ratio of GTP concentration to ATP concentration is  $\sim 0.2$  (266). With the assumption of ATP concentration in the range between 1 and 2 mM, the total GTP concentration would again be in the range of 300  $\mu\text{M}$ . The free GTP concentration is presumably at least one order of magnitude lower. Lischka and Schild (205) applied 10  $\mu\text{M}$  GTP through the patch pipette; under these approximately physiological conditions, NO did not activate  $g_{cn}$ . Unfortunately, it is not known whether CO activates  $g_{cn}$  using physiological GTP concentrations or in recordings under perforated patch conditions, nor is it established in detail whether low concentrations of cGMP ( $\sim 100 \text{ nM}$ ), which do not activate  $g_{cn}$ , affect the activation of  $g_{cn}$  by cAMP. In *Xenopus*, low cGMP concentrations prevented washout of odorant responses (205) so that there appeared to be a silent effect of cGMP on  $g_{cn}$  channels, which became operative when the cell was stimulated. The Hill coefficients of the  $g_{cn}$  channels have usually been determined with respect to either cAMP or cGMP (Table 2), whereas the physiologically relevant case, in which cGMP and cAMP act cooperatively upon  $g_{cn}$ , has so far not been analyzed.

Interestingly, the soluble guanylyl cyclase, which is coexpressed with type 2 heme oxygenase in ORNs, is not only present in cilia, but also in somata and axons (129). These findings, together with a positive correlation of CO production and high endogenous cGMP concentrations in differentiating cultured ORNs of rat, suggest an involvement of CO/cGMP signaling in developmental processes of ORNs (129).

In summary, both NO and CO have been shown to play a role in ORNs. Although the neuronal form of the NO synthase could not be demonstrated in cilia from mature ORNs using immunohistochemistry, it has been shown that some odorants stimulate increases in cGMP that are abolished by the addition of NO synthase inhibitors. Furthermore, NO blocked the washout of odorant responses and directly activated  $g_{cn}$  channels. On the other hand, heme oxygenase II has been localized in cilia, somata, and axons of ORNs. Under certain conditions, CO elicited opening of  $g_{cn}$  channels and appeared to be involved in adaptation processes. It may also be involved in the signaling during developmental processes in ORNs.

## IX. IONIC CONCENTRATIONS IN MUCUS AND ION HOMEOSTASIS

Ion concentrations in the mucus have been measured using spectrophotometry (45, 134) or ion-sensitive elec-

trodes (58, 243, 244) (Table 4). The spectrophotometric methods yield total ion concentrations, whereas ion-sensitive electrodes measure concentrations of the free ions. This may explain why Joshi et al. (134) obtained 10.7 mM as the  $\text{Ca}^{2+}$  concentration of the mucus, whereas Minor et al. (243, 244) found values in the range between 1 and 2 mM (mean, 1.5 mM) in the same species (*Rana temporaria*). The ion concentrations in frog varied during the year, being highest in February (45). Quite surprising are the high values for  $\text{K}^+$  concentrations in the mucus ( $[\text{K}^+]_{\text{muc}}$ ). Whereas in frog two reports obtained 7.9 mM (243, 244) and 10.6 mM (134), Bronstein and Leont'ev (45) obtained a  $[\text{K}^+]_{\text{muc}}$  of 69.6 mM. This difference might be explained by injured cells or cilia, which would easily increase  $[\text{K}^+]_{\text{muc}}$ . In mammals, values as high as 88 mM have been measured (45, 243), suggesting a remarkably steep  $\text{K}^+$  gradient across the tight junctions. The high  $[\text{K}^+]_{\text{muc}}$  implies equilibrium potentials for  $\text{K}^+$  that are less negative than the resting potentials. The high concentration of extracellular  $\text{K}^+$  is also typical for outer dendrites of insect ORNs (138).

What are the consequences for the transduction cascades? During odorant stimulation (using *n*-amyl acetate, geraniol, iso-eugenol,  $\alpha$ -pinene, *o*-xylene, and peppermint oil as stimuli, personal communication), Minor et al. (243, 244) obtained a decrease in  $[\text{Ca}^{2+}]_{\text{muc}}$  of  $\sim 50 \mu\text{M}$  during odorant application, which is consistent with influx of  $\text{Ca}^{2+}$  as the first electrochemical step in the transduction cascade. A change in  $[\text{Cl}^-]_{\text{muc}}$  did not exceed 0.5% during stimulation (243, 244). Whereas Joshi et al. (134), using cineole as stimulus, reported an increase in  $[\text{Na}^+]_{\text{muc}}$  during stimulation, Minor et al. (243, 244) recorded the time courses of an increase in  $[\text{K}^+]_{\text{muc}}$  and a decrease in  $[\text{Na}^+]_{\text{muc}}$ . As to the  $\text{InsP}_3/\text{Ca}^{2+}$  pathway, depolarization is brought about by the influx of cations (cf. Refs. 245, 290, 310, 311), possibly including  $\text{K}^+$  (see below). In this case,  $\text{K}^+$  would enter the cell at the mucosal side during stimula-

TABLE 4. Ion concentrations in mucus

Species	$\text{Na}^+$	$\text{K}^+$ ( $u_K$ )	$\text{Ca}^{2+}$	$\text{Cl}^-$	Reference No.
Frog					
<i>Rana temporaria</i>	142	7.9 (-63)	1.5	87	243, 244
<i>Rana temporaria</i>	52.7	10.6 (-56)	10.7		134
<i>Rana temporaria</i>	104.4	69.6 (-9)			45
Toad ( <i>Bufo marinus</i> )	85	11 (-55)	0.32	93	58
Guinea pig ( <i>C. cobaya</i> )	75.8	77.4 (-17)			45
Mouse ( <i>Mus musculus</i> )	55	57 (-24)	0.6	119	243, 244
Rat ( <i>Rattus norvegicus</i> )	122	88 (-13)	1.9	117	243, 244

Ion concentrations are given in mM.  $\text{K}^+$  potential ( $u_K$ ) is given in mV assuming intracellular  $[\text{K}^+] = 100 \text{ mM}$  (amphibians) or 150 mM (mammals).

tion, whereas  $K^+$  efflux occurs at the serosal side at rest and much more so during action potential repolarization. In addition, a  $Na^+K^+$ -ATPase localized to the cilia and the dendritic knob seems to be involved in the equilibrium of  $K^+$  (157, 209).

As to the odorant-induced activation of  $Ca^{2+}$ -dependent  $K^+$  channels (248, 249) that are localized in the transduction compartments and thought to be inhibitory, the flux through these channels depends directly on  $u_K$ . In the case of mammals, where the mucosal  $E_K$  clearly appears to be much less negative than the resting potential (Table 4), the activation of mucosal  $K^+$  channels would be excitatory. In amphibians, with  $[K^+]_{muc}$  equal to 7.9 mM (243, 244) or 10.6 mM (134),  $u_K$  is approximately at the threshold of voltage-gated  $Na^+$  channels. Whether or not spikes are generated depends on the exact values of  $u_K$  and the threshold of the  $Na^+$  conductance. If, however,  $[K^+]_{muc}$  equals 69.6 mM as measured by Bronstein and Leont'ev (45), then the  $Ca^{2+}$ -dependent  $K^+$  channels would clearly depolarize the cells and induce spiking. Because of this uncertainty, this question needs further study.

Finally, high mucosal equilibrium potentials for  $K^+$  pose, although indirectly, another question: If  $u_K > u_m$ , and  $u_{Cl} > u_m$  as reported by Dubin and Dionne (76), Kurahashi and Yau (184), and Zhainazarov and Ache (361), which conductance could be responsible for odorant-mediated inhibition, given that  $u_{Ca}$  and  $u_{Na}$  are positive?

The precise ionic composition of the mucus, although still controversial, seems thus to be of crucial importance. In most patch-clamp experiments, it is completely neglected. The *in vitro* mucosa preparation developed by Frings and Lindemann (99) leaves the polarity of the olfactory neurons intact. It thus allows investigating the function of ORNs under nearly physiological conditions. Using this preparation, Frings et al. (97) were able to establish the modulatory effects of mucosal  $Ca^{2+}$ ,  $Mg^{2+}$ ,  $Na^+$ , and  $K^+$  on forskolin-induced excitation and spiking. Whereas forskolin increased the basal spike rate, mucosal  $Ca^{2+}$  and  $Mg^{2+}$  as well as the application of *L-cis*-diltiazem lowered the spike rate. Interestingly, mucosal  $Ba^{2+}$  and 4-AP increased the spike rate, indicating the presence of apical  $K^+$  channels that render ORNs sensitive to mucosal  $K^+$ . Lowering the spike rate by removing mucosal  $Na^+$  could be counteracted by adding  $Ba^{2+}$  or 4-AP. Taken together,  $Na^+$ ,  $K^+$ ,  $Mg^{2+}$ , and  $Ca^{2+}$  all modulated the basal or the forskolin-induced activity of frog ORNs (97).

Little information is available regarding the ion homeostasis of  $Na^+$ ,  $K^+$ , and  $Ca^{2+}$  in ORNs. A  $Ca^{2+}Mg^{2+}$ -ATPase present in ORNs of salmon has been described by Lo et al. (211). This pump was shown to be thapsigargin-insensitive and particularly active in a cilia preparation with an unusually low dissociation constant of 20 nM, consistent with measured resting  $Ca^{2+}$  levels in fish ORNs (293). The function of this  $Ca^{2+}$ -ATPase is presumably  $Ca^{2+}$  extrusion from the transduction compartments (211).

Kern et al. (157) have localized the  $Na^+K^+$ -ATPase in the cilia, apical dendritic knob, and dendrite of gerbil and guinea pig ORNs, with the highest activity being localized to the cilia. In Atlantic salmon,  $Na^+K^+$ -ATPase activity has also been localized to the olfactory cilia (209). At first glance, this is an unusual localization of a  $Na^+K^+$ -ATPase, since the  $Na^+K^+$ -ATPase in epithelial cells is typically expressed at the basolateral side (12, 202). However, typically, the apical  $K^+$  concentration of epithelia is low, an exception being the high  $K^+$  concentration of the endolymph of the inner ear, maintained by the stria vascularis (359), and the extracellular medium found in insect outer dendrite (138).

The  $3Na^+/1Ca^{2+}$  exchanger has been reported to be localized primarily in the dendrite of ORNs (136). It was suggested that this localization corresponds to an uncoupling of soma and transduction compartments so that action potential-related  $Ca^{2+}$  increase in the soma would not affect processes in the dendritic knob and the cilia, and vice versa, transduction-related  $Ca^{2+}$  increases in the distal compartments of the cell would not affect  $Ca^{2+}$ -dependent processes in the soma (136).

Little is known about the role of sustentacular cells in the maintenance of ion homeostasis in the mucus, although from parallels with other epithelia, it is likely that these cells play an important role. Persaud et al. (272) found that basal ion transport across frog olfactory mucosa was inhibited by apically applied furosemide, but not by amiloride. They suggested that a furosemide-sensitive  $Na$ - $Cl$  or  $Na$ - $K$ - $Cl$  cotransport system may exist in the apical membranes of sustentacular cells. In addition, these authors found that  $K^+$  pathways are available on the apical side of the mucosa. They speculated the existence of  $K^+$  channels on the apical membranes of the sustentacular and/or olfactory receptor cells. Further work is necessary to determine the precise role of sustentacular cells in mucosal ion homeostasis.

In summary, the ion concentrations in the mucus of the olfactory mucosa have as yet received relatively little attention, although they obviously may affect the responses to odorants crucially. The ion composition of the mucosa is similar to that of the extracellular space, the important exceptions being  $[Ca^{2+}]_{muc}$  which seems to be slightly lower and  $[K^+]_{muc}$  which, at least in some species, is markedly higher than in the extracellular fluid. The resulting elevated equilibrium potential of  $K^+$  would mean that  $Ca^{2+}$ -dependent  $K^+$  currents would be depolarizing. The concentration gradients are maintained by the action of ion pumps and exchangers. A  $Ca^{2+}$ -ATPase and the  $3Na^+-2K^+$ -ATPase have been reported to be expressed in the transduction compartments of ORNs, whereas the  $3Na^+/1Ca^{2+}$  exchanger appears to be localized mainly on the dendritic compartment.

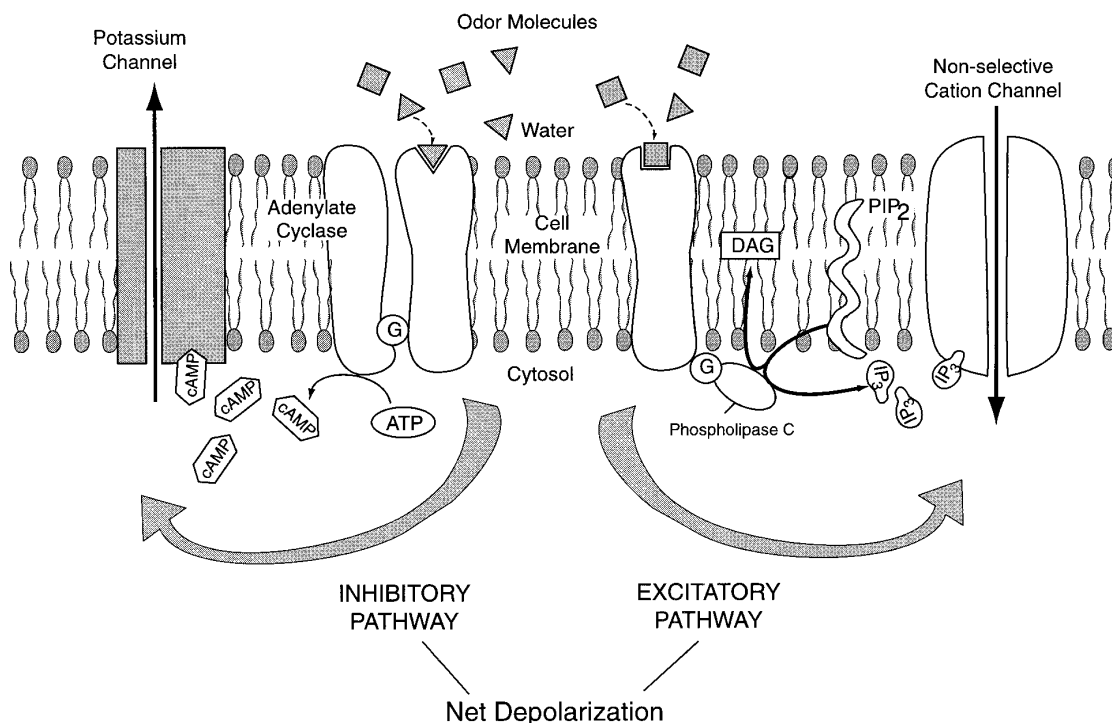


FIG. 9. Working model of dual olfactory transduction pathways in olfactory receptor neurons of spiny lobster, *Panulirus argus*. *Top*: odorants activate either a cAMP-mediated cascade including receptors, G protein, and adenylyl cyclase, or an  $\text{InsP}_3$ -mediated pathway, including receptors, G protein, and phospholipase C. In the former case, cAMP directly gates  $\text{K}^+$  channels leading to a hyperpolarization (*bottom left*), whereas  $\text{InsP}_3$  activates nonselective cation channels, thereby causing a depolarizing receptor potential (*bottom right*). [From Ache (2).]

## X. MULTIPLE PATHWAYS: IMPLICATIONS FOR OLFACTORY CODING

In view of the current controversy about the existence of multiple second messenger pathways (see sects. *IV D* and *VI B 4*), it is instructive to consider the consequences of the existence of one or multiple second messenger pathways for odorant quality coding.

Information on odorant quality, which is directly related to the chemical structure of the odorants, is conveyed to the olfactory bulb by the ORNs through synaptic connections with mitral/tufted and periglomerular cells (84). In the simplest scheme, individual ORNs could express a single olfactory receptor per cell which, when occupied by an appropriate odorant, would stimulate a single second messenger pathway, thereby eliciting a generator potential that would increase the neuron's firing rate. Such a model has been suggested on the basis of in situ hybridization studies that show that each olfactory neuron expresses one or a few putative olfactory receptors that project to one or a few glomeruli (191, 258, 261, 285, 286, 348, 349) and electrophysiological and molecular biological studies that favor the role of cAMP as the sole second messenger in olfactory transduction (50, 256, 257). In this model, the second messenger system could be viewed as a black box that would simply serve the role of

faithfully transducing information on receptor occupancy into an increase in the frequency of action potential firing. In this case, the only relevance of the study of the transduction mechanisms in the olfactory receptor cell for odor quality coding would be the exact determination of the function relating receptor occupancy to the output of the cell. Other details of peripheral olfactory transduction in the ORN would be irrelevant to quality coding.

In the case of multiple second messenger pathways, peripheral transduction can still be considered a black box if each olfactory neuron expresses only one pathway. In this case, the only change is that there will be a number of black box types (one for each pathway). Otherwise, the implications for olfactory coding remain the same as for a single second messenger pathway.

The situation is quite different if two olfactory receptors are expressed in a single ORN as suggested by electrophysiological experiments (27, 75, 142, 146, 245, 249, 291) and as shown elegantly by genetic manipulations in *C. elegans* (21, 320, 321, 341), especially if the two second messenger pathways are coexpressed in a single cell as demonstrated or suggested by various electrophysiological studies (27, 75, 142, 146, 245, 249, 291). If stimulation of second messenger formation in olfactory neurons could lead to depolarization or hyperpolarization depending on which second messenger-regulated conductances are acti-

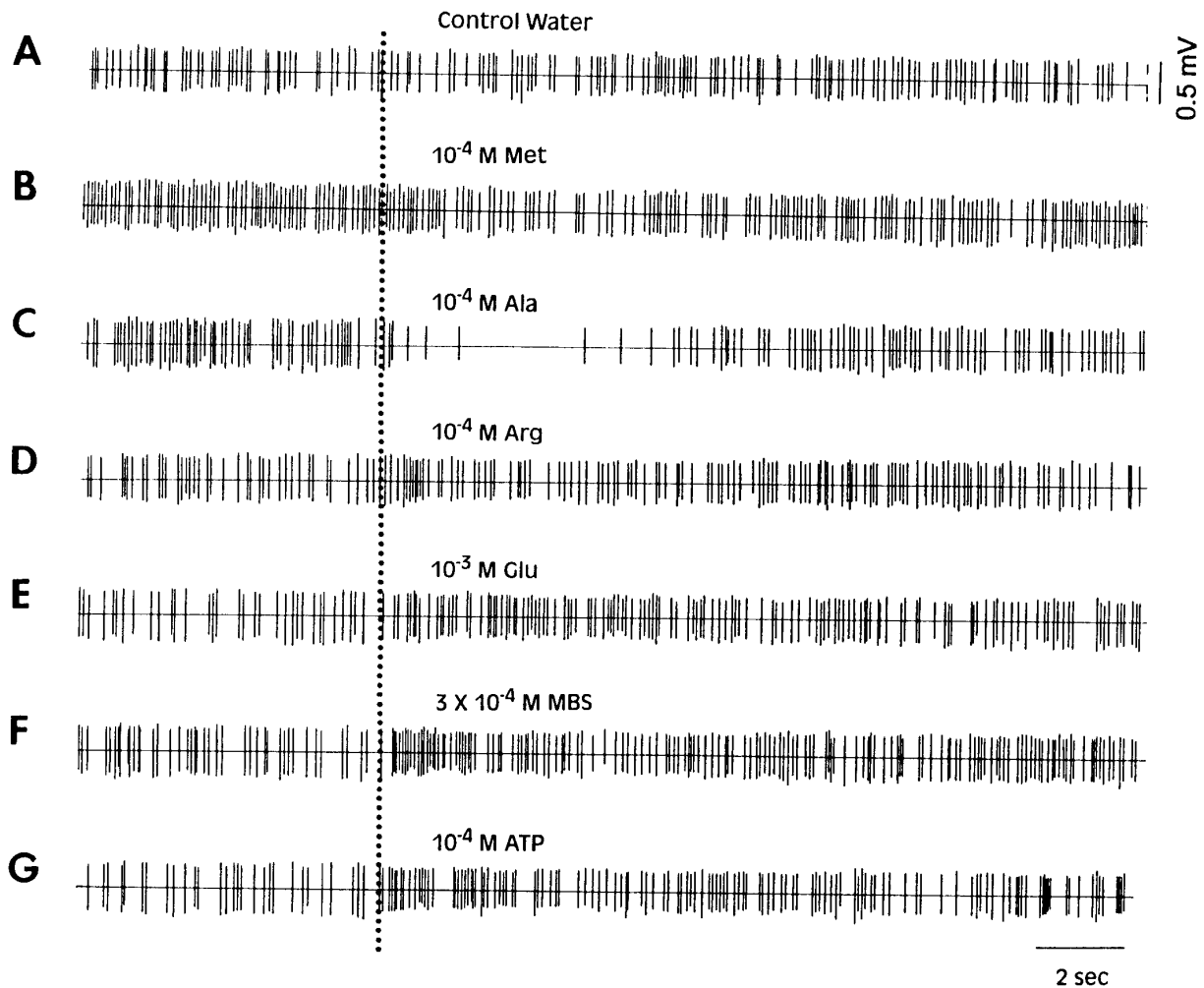


FIG. 10. Representative responses of single olfactory receptor neurons of channel catfish, *Ictalurus punctatus*, to 6 stimuli and to a water control. *A*: no significant change from spontaneous activity to water control. *B*: suppressive response to  $10^{-4}$  M methionine (Met). *C*: suppressive response to  $10^{-4}$  M alanine (Ala). *D*: excitatory response to  $10^{-4}$  M arginine (Arg). *E*: excitatory response to  $10^{-3}$  M glutamic acid (Glu). *F*: excitatory response to  $3 \times 10^{-4}$  M MBS (sodium salts of cholic acid, taurocholic acid, and tauroolithocholic acid each at  $10^{-4}$  M). *G*: excitatory response to  $10^{-4}$  M ATP. Vertical dotted line indicates beginning of neural responses as defined by onset of simultaneously recorded electroolfactogram response. Both excitatory and suppressive responses recovered to spontaneous level of activity. [Modified from Kang and Caprio (142).]

vated in a given neuron (27, 105, 108, 109, 142, 249), or if the two pathways interact with each other, then interactions among second messenger pathways in the periphery could contribute to coding of odor quality information. Figure 9 depicts a working model of a dual olfactory transduction pathway in the lobster, where fish food led to a depolarizing response via activation of a cation conductance; the depolarizing response was, however, short-circuited by an odorant-activated  $K^+$  conductance (2, 240a). Figure 10 gives an example of a catfish ORN that clearly responds differentially to different stimuli (142). As suggested by Ache (2) and Dionne and Dubin (74), the ability of the primary receptor neurons in the olfactory system to respond with excitation or inhibition would enhance the ability of the olfactory system to discriminate between

odorants, because a complex odor could be represented as more than simply the sum of the components. Cells that respond to one molecular determinant with suppression and to another with excitation may play a role in enhancing contrast between odorants that share partial structural homology.

Differential stimulation or suppression of olfactory neurons by odors could be used by the olfactory system as a mechanism for contrast enhancement. In addition, the responses resulting from simultaneous stimulation and inhibition of different neurons by one odorant could be contrasted in the olfactory bulb in such a way that low odorant concentrations could be detected at signal levels that could not be resolved from noise in a purely excitatory system.

In summary, although it has not yet been shown conclusively that peripheral olfactory transduction plays a role in quality coding in the olfactory system, it is likely that second messenger pathways do not simply play the role of a black box as far as quality coding is concerned. Multiple olfactory transduction pathways could lead to increased odorant discriminability and detectability.

## XI. SUMMARY AND PERSPECTIVES

Our understanding of the molecular mechanisms of olfactory transduction has advanced greatly during the last decade because of the application of modern techniques of molecular biology, electrophysiology, and biophysics to the study of ORNs. First proposed to be the second messenger mediating olfactory transduction on the basis of biochemical studies, cAMP was shown to directly gate nonspecific cation channels. The evidence supporting a mediatory role for these cAMP-gated channels in olfactory transduction is conclusive. In addition, the influx of  $\text{Ca}^{2+}$  through cAMP-gated channels elicits an increase in  $[\text{Ca}^{2+}]_i$  leading to opening of  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels. Although the precise nature of the regulation of intracellular  $\text{Cl}^-$  in olfactory neurons must be addressed in future studies, it is clear that the  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels participate in the transduction cascade by amplifying the response.

However, studies carried out throughout the last decade also indicated that cAMP is not the only second messenger involved in olfactory transduction and confirmed early reports of inhibitory odorant responses. In addition, there is a considerable amount of evidence indicating that  $\text{InsP}_3$  plays a role in olfactory transduction. Although the precise nature of the involvement of  $\text{InsP}_3$  in olfactory transduction remains controversial, the discovery of plasma membrane ion channels activated by  $\text{InsP}_3$  suggests that this second messenger may mediate olfactory responses for some odorants. Future work must determine whether  $\text{InsP}_3$  is indeed a second messenger mediating responsiveness to odors, or whether it plays a modulatory role. In particular, detailed work by different laboratories using the same animal model systems must determine whether apparently discrepant observations on this alternate second messenger pathway are because of species differences or differences in experimental conditions.

The finding that different odorants can cause either excitation or suppression of action potential firing in the same olfactory neuron is exciting because it opens the possibility that peripheral olfactory transduction plays a role in odor quality coding. Future studies must seek to conclusively determine the molecular events underlying odorant-induced suppression of action potential firing. The role, if any, of peripheral olfactory transduction in odor quality coding must be qualified.

Experiments carried out in the last decade have established a role for  $\text{Ca}^{2+}$  as a "third messenger" in olfactory transduction. The influx of  $\text{Ca}^{2+}$  elicits amplification of the odor-induced current through opening of  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels and participates in adaptation during sustained stimulation. Both the cAMP-gated channel and the two  $\text{InsP}_3$ -regulated conductances are permeable for  $\text{Ca}^{2+}$ , and opening of these channels causes a spatially restricted increase in intracellular  $\text{Ca}^{2+}$ . This increase in  $[\text{Ca}^{2+}]_i$  elicits opening of  $\text{Cl}^-$  or nonspecific cation channels amplifying the response to odorants and may elicit opening of  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels. In addition, other actions have been described for the increase in  $[\text{Ca}^{2+}]_i$ , including inhibition of the activity of the cAMP-gated channel through calmodulin, modulation of  $\text{InsP}_3$ -regulated conductances, regulation of the actions of CO and NO, and modulation of enzymatic activity. Consistent with the complex action of  $[\text{Ca}^{2+}]_i$ , it has been determined that  $[\text{Ca}^{2+}]_i$  is tightly regulated in a compartmentalized manner within ORNs. The understanding of  $\text{Ca}^{2+}$  homeostasis and of the regulatory action of  $[\text{Ca}^{2+}]_i$  is incomplete, and future experiments must address this issue to obtain a better understanding of olfactory transduction.

Studies of olfactory transduction during the last decade have concentrated on the function of isolated ORNs, but little is known about the mechanisms of olfactory transduction in ORNs in situ. Studies at the cellular level with more intact in vitro systems in which the integrity of the neuroepithelium is conserved are needed to clarify the role of proposed intercellular communication through molecules such as CO and NO and of efferent control of the function of ORNs. In addition, because ion concentrations in the mucus affect the reversal potential of odorant-regulated channels in the cilia, and the presence of odorant binding proteins in the mucus may affect the interaction of odorants with receptors, determination of the mechanisms for ion homeostasis and odorant delivery in the mucus overlaying the olfactory epithelium is crucial to the complete understanding of olfactory transduction.

In conclusion, a review of the literature on olfactory transduction in the last decade reveals unprecedented progress in the study of olfactory transduction. Previously intractable questions about signal transduction in ORNs have been answered. As a result, not only has a better understanding of olfactory transduction been obtained, but the study of olfactory transduction has provided new findings relevant for understanding the mechanisms of signal transduction in other types of neurons and sensory receptor cells. The olfactory receptor cell might even turn out to be the first neuron that can be completely characterized in terms of signal input, second messenger processing, and output generation. A survey of the literature also reveals the fact that many questions remain unanswered. It is likely that the next decade of research in olfactory transduction will be just as prolific as the last,

resulting in a deeper understanding of intra- and intercellular interactions within and among ORNs.

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## REFERENCES

1. ABOGADIE, F. C., R. C. BRUCH, AND A. I. FARBMAN. G-protein subunits expressed in catfish olfactory receptor neurons. *Chem. Senses* 20: 199–206, 1995.
2. ACHE, B. W. Towards a common strategy for transducing olfactory information. *Semin. Cell Biol.* 5: 55–63, 1994.
3. ACHE, B. W., AND A. B. ZHAINAZAROV. Dual second-messenger pathways in olfactory transduction. *Curr. Opin. Neurobiol.* 5: 461–466, 1995.
4. ADAMEK, G. D., AND R. C. GESTELAND. A saline-filled pipette that records olfactory receptor cell activity. *Chem. Senses* 9: 121–126, 1984.
5. ALTENHOFEN, W., J. LUDWIG, E. EISMANN, W. KRAUS, W. BONGK, AND U. B. KAUPP. Control of ligand-specificity in cyclic nucleotide-gated channels from rod photoreceptors and olfactory epithelium. *Proc. Natl. Acad. Sci. USA* 88: 9868–9872, 1991.
6. ALTNER, H., AND W. MÜLLER. Elektrophysiologische und elektronmikroskopische Untersuchungen an der Riechschleimhaut des Jacobsonschen Organs von Eidechsen (*Lacerta*). *Z. Vergl. Physiol.* 60: 151–155, 1968.
7. ANDERSON, P. A. V., AND B. W. ACHE. Voltage- and current-clamp recordings of the receptor potential in olfactory receptor cells in situ. *Brain Res.* 338: 273–280, 1985.
8. ANDERSON, P. A. V., AND K. A. HAMILTON. Intracellular recordings from isolated salamander olfactory receptor neurons. *Neuroscience* 21: 167–173, 1987.
9. ANHOLT, R. R. H. Molecular neurobiology of olfaction. *Crit. Rev. Neurobiol.* 7: 1–22, 1993.
10. ANHOLT, R. R. H. Signal integration in the nervous system: adenylate cyclases as molecular coincidence detectors. *Trends Neurosci.* 17: 37–41, 1994.
11. ANHOLT, R. R. H., U. AEBI, AND S. H. SNYDER. A partially purified preparation of isolated chemosensory cilia from the olfactory epithelium of the bullfrog, *Rana catesbeiana*. *J. Neurosci.* 6: 1962–1969, 1986.
12. APPELL, H.-J. Electrogenic properties of the Na<sup>+</sup>,K<sup>+</sup> pump. *J. Membr. Biol.* 110: 103–114, 1989.
13. ARMSTRONG, C. M., AND W. F. GILLY. Access resistance and space clamp problems associated with whole-cell patch clamping. *Methods Enzymol.* 207: 100–122, 1992.
14. ARMSTRONG, D. L. Calcium channel regulation by calcineurin, a Ca<sup>2+</sup>-activated phosphatase in mammalian brain. *Trends Neurosci.* 12: 117–123, 1989.
15. AVENET, P., F. HOFMANN, AND B. LINDEMANN. Transduction in taste receptor cells requires cAMP-dependent protein kinase. *Nature* 331: 351–354, 1988.
16. BACIGALUPO, J., B. MORALES, P. LABARCA, AND R. MADRID. Involvement of Ca<sup>2+</sup>-activated K<sup>+</sup> channel in odorant-triggered inhibitory responses of toad olfactory neurons (Abstract). *Chem. Senses* 20: 661, 1995.
17. BAKALYAR, H. A., AND R. R. REED. Identification of a specialized adenylate cyclase that may mediate odorant detection. *Science* 250: 1403–1406, 1990.
18. BALASUBRAMANIAN, S., G. LYNCH, AND P. H. BARRY. Calcium-dependent modulation of the agonist affinity of the mammalian cyclic nucleotide-gated channel by calmodulin and a novel endogenous factor. *J. Membr. Biol.* 152: 13–23, 1996.
19. BALASUBRAMANIAN, S., J. W. LYNCH, AND P. H. BARRY. The permeation of organic cations through cAMP-gated channels in mammalian olfactory receptor neurons. *J. Membr. Biol.* 146: 177–191, 1995.
20. BANNISTER, L. H. The fine structure of the sensory endings in the vomeronasal organ of the slow worm *Anguis fragilis*. *Nature* 217: 275–276, 1968.
21. BARGMANN, C. I., E. HARTWIEG, AND H. R. HORVITZ. Odorant-selective genes and neurons mediate olfaction in *C. elegans*. *Cell* 74: 515–527, 1993.
22. BAUMANN, A., S. FRINGS, M. GODDE, R. SEIFERT, AND U. B. KAUPP. Primary structure and functional expression of a *Drosophila* cyclic nucleotide-gated channel present in eyes and antennae. *EMBO J.* 13: 5040–5050, 1994.
23. BAYLOR, D. A., T. D. LAMB, AND K.-W. YAU. Responses of retinal rods to single photons. *J. Physiol. (Lond.)* 288: 613–634, 1979.
24. BERGHARD, A., L. B. BUCK, AND E. R. LIMAN. Evidence for distinct signaling mechanisms in two mammalian olfactory sense organs. *Proc. Natl. Acad. Sci. USA* 93: 2365–2369, 1996.
25. BÉHÉ, P., J. A. DESIMONE, P. AVENET, AND B. LINDEMANN. Membrane currents in taste cells of the rat fungiform papilla: evidence for two types of Ca currents and inhibition of K currents by saccharin. *J. Gen. Physiol.* 96: 1061–1084, 1990.
26. BOEKHOFF, I., AND H. BREER. Termination of second messenger signaling in olfaction. *Proc. Natl. Acad. Sci. USA* 89: 471–474, 1992.
27. BOEKHOFF, I., W. C. MICHEL, H. BREER, AND B. W. ACHE. Single odors differentially stimulate dual second messenger pathways in lobster olfactory receptor cells. *J. Neurosci.* 14: 3304–3309, 1994.
28. BOEKHOFF, I., E. TAREILUS, J. STROTMANN, AND H. BREER. Rapid activation of alternative second messenger pathways in olfactory cilia from rats by different odorants. *EMBO J.* 9: 2453–2458, 1990.
29. BORISY, F. F., P. N. HWANG, G. V. RONNETT, AND S. H. SNYDER. High-affinity cAMP phosphodiesterase and adenosine localized in sensory organs. *Brain Res.* 610: 199–207, 1993.
30. BORISY, F. F., G. V. RONNETT, A. M. CUNNINGHAM, D. JULFS, AND J. BEAVO. Calcium/calmodulin-activated phosphodiesterase expressed in olfactory receptor neurons. *J. Neurosci.* 12: 915–923, 1992.
31. BORMANN, J. Electrophysiology of GABA<sub>A</sub> and GABA<sub>B</sub> receptor subtypes. *Trends Neurosci.* 11: 112–116, 1988.
32. BOUVET, J. F., J. C. DELALEU, AND A. HOLLEY. Olfactory receptor cell function is affected by trigeminal nerve activity. *Neurosci. Lett.* 77: 181–186, 1987.
33. BOUVET, J. F., J. C. DELAULEU, AND A. HOLLEY. The activity of olfactory receptor cells is affected by acetylcholine and substance P. *Neurosci. Res.* 5: 214–223, 1988.
34. BOYLE, A. G., Y. S. PARK, T. HUQUE, AND R. C. BRUCH. Properties of phospholipase C in isolated olfactory cilia from the channel catfish (*Ictalurus punctatus*). *Comp. Biochem. Physiol. B Biochem.* 88: 767–775, 1987.
35. BRADLEY, J., J. LI, N. DAVIDSON, H. A. LESTER, AND K. ZINN. Heteromeric olfactory cyclic nucleotide-gated channels: a subunit that confers increased sensitivity to cAMP. *Proc. Natl. Acad. Sci. USA* 91: 8890–8894, 1994.
36. BREEDT, D. S., AND S. H. SNYDER. Nitric oxide: a physiologic messenger molecule. *Annu. Rev. Biochem.* 63: 175–195, 1994.
37. BREER, H., AND I. BOEKHOFF. Odorants of the same odor class activate different second messenger pathways. *Chem. Senses* 16: 19–29, 1991.
38. BREER, H., I. BOEKHOFF, AND E. TAREILUS. Rapid kinetics of second messenger formation in olfactory transduction. *Nature* 345: 65–68, 1990.
39. BREER, H., T. KLEMM, AND I. BOEKHOFF. Nitric oxide mediated formation of cyclic GMP in the olfactory system. *Neuroreport* 3: 1030–1032, 1992.
40. BREER, H., K. RAMING, AND J. KRIEGER. Signal recognition and transduction in olfactory neurons. *Biochim. Biophys. Acta* 1224: 277–287, 1994.
41. BREER, H., AND G. M. SHEPHERD. Implications of the NO/cGMP system for olfaction. *Trends Neurosci.* 16: 5–9, 1993.

42. BROILLET, M. C., AND S. FIRESTEIN. Direct activation of the olfactory cyclic nucleotide-gated channel through modification of sulfhydryl groups by NO compounds. *Neuron* 16: 377–385, 1996.
43. BROILLET, M. C., AND S. FIRESTEIN. Gaseous second messengers in vertebrate olfaction. *J. Neurobiol.* 30: 49–57, 1996.
44. BROILLET, M. C., AND S. FIRESTEIN.  $\beta$ -Subunits of the olfactory cyclic nucleotide-gated channel form a nitric oxide activated  $Ca^{2+}$  channel. *Neuron*. 18: 951–958, 1997.
45. BRONSTEIN, A. A., AND V. G. LEONTEV. The sodium and potassium content of the mucus of the olfactory epithelium of vertebrates. *J. Evol. Biochem. Physiol.* 8: 520–524, 1972.
46. BRUCH, R. C. Signal transducing GTP-binding proteins in olfaction. *Comp. Biochem. Physiol. A Physiol.* 95: 27–29, 1996.
47. BRUCH, R. C. Phosphoinositide second messengers in olfaction. *Comp. Biochem. Physiol. B Biochem.* 113: 451–459, 1996.
48. BRUCH, R. C., AND T. HUQUE. Odorant- and guanine nucleotide-stimulated phosphoinositide turnover in olfactory cilia (Abstract). *Chem. Senses* 11: 585, 1986.
49. BRUCH, R. C., AND J. H. TEETER. Cyclic AMP links amino acid chemoreceptors to ion channels in olfactory cilia. *Chem. Senses* 15: 419–430, 1990.
50. BRUNET, L. J., G. H. GOLD, AND J. NGAI. General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. *Neuron* 17: 681–693, 1996.
51. BUCK, L., AND R. AXEL. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* 65: 175–187, 1991.
52. BUCK, L. B. Information coding in the vertebrate olfactory system. *Annu. Rev. Neurosci.* 19: 517–544, 1996.
53. CAPRIO, J., AND R. P. BYRD, JR. Electrophysiological evidence for acidic, basic and neutral amino acid olfactory receptor sites in catfish. *J. Gen. Physiol.* 84: 403–422, 1984.
54. CARR, V. M., A. I. FARBMAN, L. M. COLETTI, AND J. I. MORGAN. Identification of a new non-neuronal cell type in rat olfactory epithelium. *Neuroscience* 45: 433–449, 1996.
55. CASTLE, N. A., D. G. HAYLETT, AND D. H. JENKINSON. Toxins in the characterization of potassium channels. *Trends Neurosci.* 12: 59–65, 1989.
56. CHEN, T.-Y., AND K.-W. YAU. Direct modulation by  $Ca^{2+}$ -calmodulin of cyclic nucleotide-activated channel of rat olfactory receptor neurons. *Nature* 368: 545–548, 1994.
57. CHEN, Z., U. PACE, J. HELDMAN, A. SHAPIRA, AND D. LANCET. Isolated frog olfactory cilia: a preparation of dendritic membranes from chemosensory neurons. *J. Neurosci.* 6: 2146–2154, 1986.
58. CHIU, D., T. NAKAMURA, AND G. H. GOLD. Ionic composition of toad mucus measured with ion selective microelectrodes. *Chem. Senses* 13: 677–678, 1988.
59. CINELLI, A. R., K. A. HAMILTON, AND J. S. KAUER. Salamander olfactory bulb neuronal activity observed by video rate, voltage-sensitive dye imaging. III. Spatial and temporal properties of responses evoked by odorant stimulation. *J. Neurophysiol.* 73: 2053–2071, 1995.
60. CINELLI, A. R., AND J. S. KAUER. Voltage-sensitive dyes and functional activity in the olfactory pathway. *Annu. Rev. Neurosci.* 15: 321–351, 1994.
61. CINELLI, A. R., AND J. S. KAUER. Salamander olfactory bulb neuronal activity observed by video rate, voltage-sensitive dye imaging. II. Spatial and temporal properties of responses evoked by electric stimulation. *J. Neurophysiol.* 73: 2033–2052, 1995.
62. CINELLI, A. R., S. R. NEFF, AND J. S. KAUER. Salamander olfactory bulb neuronal activity observed by video rate, voltage-sensitive dye imaging. I. Characterization of the recording system. *J. Neurophysiol.* 73: 2017–2032, 1995.
63. COBURN, C. M., AND C. I. BARGMANN. A putative cyclic nucleotide-gated channel is required for sensory development and function in *C. elegans*. *Neuron* 17: 695–706, 1996.
64. CONNOR, J. A., AND C. F. STEVENS. Voltage clamp studies of a transient outward membrane current in gastropod neural somata. *J. Physiol. (Lond.)* 213: 21–30, 1971.
65. COROTTO, F. S., D. R. PIPER, N. CHEN, AND W. C. MICHEL. Voltage- and  $Ca^{2+}$ -gated currents in zebrafish olfactory receptor neurons. *J. Exp. Biol.* 199: 1115–1126, 1996.
66. CUMMINGS, T. A., J. POWELL, AND S. C. KINNAMON. Sweet taste transduction in hamster taste cells: evidence for the role of cyclic nucleotides. *J. Neurophysiol.* 70: 2326–2336, 1993.
67. CUNNINGHAM, A. M., D. K. RYUGO, A. H. SHARP, R. R. REED, S. H. SNYDER, AND G. V. RONNETT. Neuronal inositol 1,4,5-trisphosphate receptor localized to the plasma membrane of olfactory cilia. *Neuroscience* 57: 339–352, 1993.
68. DAWSON, T. M., J. L. ARRIZA, D. E. JAWORSKY, F. BORISY, H. ATTRAMADAL, R. J. LEFKOWITZ, AND G. V. RONNETT. Beta-adrenergic receptor kinase-2 and beta-arrestin-2 as mediators of odorant-induced desensitization. *Science* 259: 825–829, 1993.
69. DELGADO, R., AND P. LABARCA. Properties of whole cell currents in isolated olfactory neurons from the Chilean toad *Caudiverbera caudiverbera*. *Am. J. Physiol.* 264 (Cell Physiol. 33): C1418–C1427, 1993.
70. DELLACORTE, C., D. L. KALLINOSKI, T. HUQUE, L. WYSOCKI, AND D. RESTREPO. NADPH diaphorase staining suggests localization of nitric oxide synthase within vertebrate olfactory neurons. *Neuroscience* 66: 215–225, 1995.
71. DELLACORTE, C., D. RESTREPO, B. P. M. MENDO, I. ANDREINI, AND D. L. KALLINOSKI.  $G_{\alpha q}/G_{\alpha 11}$ : immunolocalization in the neuroepithelium of the rat (*Rattus rattus*) and the channel catfish (*Ictalurus punctatus*). *Neuroscience* 74: 261–273, 1996.
72. DHALLAN, R. S., K. YAU, K. A. SCHRADER, AND R. R. REED. Primary structure and functional expression of a cyclic nucleotide-activated channel from olfactory neurons. *Nature* 347: 184–187, 1990.
73. DIONNE, V. E. Chemosensory responses in isolated olfactory receptor neurons from *Necturus maculosus*. *J. Gen. Physiol.* 99: 415–433, 1992.
74. DIONNE, V. E., AND A. E. DUBIN. Transduction diversity in olfaction. *J. Exp. Biol.* 194: 1–21, 1994.
75. DUBIN, A. E., AND V. E. DIONNE. Modulation of  $Cl^-$ ,  $K^+$ , and nonselective cation conductances by taurine in olfactory receptor neurons of the mudpuppy *Necturus maculosus*. *J. Gen. Physiol.* 101: 469–485, 1993.
76. DUBIN, A. E., AND V. E. DIONNE. Action potentials and chemosensitive conductance in the dendrites of olfactory neurons suggest new features for odor transduction. *J. Gen. Physiol.* 103: 181–201, 1994.
77. DULAC, C., AND R. AXEL. A novel family of genes encoding putative pheromone receptors in mammals. *Cell* 83: 195–206, 1995.
78. ERICKSON, J. R., AND J. CAPRIO. The spatial distribution of ciliated and microvillous olfactory receptor neurons in the channel catfish is not matched by a differential specificity to amino acid and bile salt stimuli. *Chem. Senses* 9: 127–139, 1984.
79. EXTON, J. H. Role of G proteins in activation of phosphoinositide phospholipase C. *Adv. Second Messenger Phosphoprotein Res.* 28: 65–72, 1993.
80. FADDOOL, D. A., AND B. W. ACHE. Plasma membrane inositol 1,4,5-trisphosphate-activated channels mediate signal transduction in lobster olfactory receptor neurons. *Neuron* 9: 907–918, 1992.
81. FADDOOL, D. A., AND B. W. ACHE. Inositol 1,3,4,5-tetrakisphosphate-gated channels interact with inositol 1,4,5-trisphosphate-gated channels in olfactory receptor neurons. *Proc. Natl. Acad. Sci. USA* 91: 9471–9475, 1994.
82. FADDOOL, D. A., S. J. ESTEY, AND B. W. ACHE. Evidence that a  $G_q$  protein mediates excitatory odor transduction in lobster olfactory receptor neurons. *Chem. Senses* 20: 489–498, 1995.
83. FADDOOL, D. A., W. C. MICHEL, AND B. W. ACHE. Odor sensitivity of cultured lobster olfactory receptor neurons is not dependent on process formation. *J. Exp. Biol.* 174: 215–233, 1993.
84. FARBMAN, A. I. *Cell Biology of Olfaction*. New York: Cambridge Univ. Press, 1992.
85. FESENKO, E. E., V. I. NOVOSELOV, G. Y. PERVUKHIN, AND N. K. FESENKO. Molecular mechanisms of odor sensing. II. Studies of fractions from olfactory tissue scrapings capable of sensitizing artificial lipid membranes to action of odorants. *Biochim. Biophys. Acta* 466: 347–356, 1996.
86. FIRESTEIN, S., B. DARROW, AND G. M. SHEPHERD. Activation of the sensory current in salamander olfactory receptor neurons depends on a G protein-mediated cAMP second messenger system. *Neuron* 6: 825–835, 1991.
87. FIRESTEIN, S., C. PICCO, AND A. MENINI. The relation between

- stimulus and response in olfactory receptor cells of the tiger salamander. *J. Physiol. (Lond.)* 468: 1–10, 1993.
88. FIRESTEIN, S., AND G. M. SHEPHERD. Neurotransmitter antagonists block some odor responses in olfactory receptor neurons. *Neuroreport* 3: 661–664, 1992.
  89. FIRESTEIN, S., AND G. M. SHEPHERD. Interaction of anionic and cationic currents leads to a voltage dependence in the odor response of olfactory receptor neurons. *J. Neurophysiol.* 73: 562–567, 1995.
  90. FIRESTEIN, S., G. M. SHEPHERD, AND F. S. WERBLIN. Time course of the membrane current underlying sensory transduction in salamander olfactory receptor neurones. *J. Physiol. (Lond.)* 430: 135–158, 1990.
  91. FIRESTEIN, S., AND F. WERBLIN. Odor-induced membrane currents in vertebrate-olfactory receptor neurons. *Science* 244: 79–82, 1989.
  92. FIRESTEIN, S., AND F. S. WERBLIN. Gated currents in isolated olfactory receptor neurons of the larval tiger salamander. *Proc. Natl. Acad. Sci. USA* 84: 6292–6296, 1987.
  93. FIRESTEIN, S., F. ZUFALL, AND G. M. SHEPHERD. Single odor-sensitive channels in olfactory receptor neurons are also gated by cyclic nucleotides. *J. Neurosci.* 11: 3565–3572, 1991.
  94. FRANCESCHINI, V., P. P. GIORGI, AND F. CIANI. Primary olfactory terminations in the forebrain of amphibia: a comparative study with soybean agglutinin. *J. Hirnforsch.* 6: 627–635, 1992.
  95. FRANCESCHINI, V., M. LAZZARI, AND F. CIANI. Identification of surface glycoconjugates in the olfactory system of turtle. *Brain Res.* 725: 81–87, 1996.
  96. FRINGS, S. Protein kinase C sensitizes olfactory adenylate cyclase. *J. Gen. Physiol.* 101: 183–205, 1993.
  97. FRINGS, S., S. BENZ, AND B. LINDEMANN. Current recording from sensory cilia of olfactory receptor cells in situ. II. Role of mucosal  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Ca}^{2+}$  ions. *J. Gen. Physiol.* 97: 725–747, 1991.
  98. FRINGS, S., AND B. LINDEMANN. Odorant response of isolated olfactory receptor cells is blocked by amiloride. *J. Membr. Biol.* 105: 233–243, 1988.
  99. FRINGS, S., AND B. LINDEMANN. Single unit recording from olfactory cilia. *Biophys. J.* 57: 1091–1094, 1990.
  100. FRINGS, S., AND B. LINDEMANN. Current recording from sensory cilia of olfactory receptor cells in situ. I. The neuronal response to cyclic nucleotides. *J. Gen. Physiol.* 97: 1–16, 1991.
  101. FRINGS, S., J. W. LYNCH, AND B. LINDEMANN. Properties of cyclic nucleotide-gated channels mediating olfactory transduction. Activation, selectivity, and blockage. *J. Gen. Physiol.* 100: 45–67, 1992.
  102. FRINGS, S., R. SEIFERT, M. GODDE, AND U. B. KAUPP. Profoundly different calcium permeation and blockage determine the specific function of distinct cyclic-nucleotide-gated channels. *Neuron* 15: 169–179, 1995.
  103. GARTHWAITE, J. Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci.* 14: 60–67, 1991.
  104. GESTELAND, R. C. Neural coding in olfactory receptor cells. In: *Handbook of Sensory Physiology. Chemical Senses*, edited by L. M. Beidler. Berlin: Springer-Verlag, 1971, p. 132–150.
  105. GESTELAND, R. C., J. Y. LETTVIN, AND W. H. PITTS. Chemical transmission in the nose of the frog. *J. Physiol. (Lond.)* 181: 525–559, 1965.
  106. GETCHELL, T. V. Functional properties of vertebrate olfactory receptor neurons. *Physiol. Rev.* 66: 772–818, 1986.
  107. GETCHELL, T. V., F. L. MARGOLIS, AND M. GETCHELL. Perireceptor and receptor events in vertebrate olfaction. *Prog. Neurobiol.* 23: 317–345, 1984.
  108. GETCHELL, T. V., AND G. M. SHEPHERD. Responses of olfactory receptor cells to step pulses of odour at different concentrations in the salamander. *J. Physiol. (Lond.)* 282: 521–540, 1978.
  109. GETCHELL, T. V., AND G. M. SHEPHERD. Adaptive properties of olfactory receptors analysed with odour pulses of varying durations. *J. Physiol. (Lond.)* 282: 541–560, 1978.
  110. GOMEZ, G., D. RESTREPO, N. RAWSON, L. D. LOWRY, W. M. KEANE, AND J. L. ROTHSTEIN. Induction of differentiation of human olfactory neuroblastoma cells into odorant responsive cells. *Neuroscience* 74: 567–577, 1996.
  111. GOULDING, E. H., J. NGAI, R. H. KRAMER, S. COLICOS, R. AXEL, S. A. SIEGELBAUM, AND A. CHESS. Molecular cloning and single-channel properties of the cyclic nucleotide-gated channel from catfish olfactory neurons. *Neuron* 8: 45–58, 1992.
  112. GRAZIADEI, P. P. C., AND D. TUCKER. Vomeronasal receptors in turtles. *Z. Zellforsch.* 105: 498–514, 1970.
  113. GREGER, R. Cellular membrane transport mechanisms. In: *Comprehensive Human Physiology: From Cellular Mechanisms to Integration*, edited by R. Greger and U. Windhorst. Berlin: Springer, 1996, p. 149–171.
  114. GRIFFIN, H. D., M. SYKES, AND J. N. HAWTHORNE. Effects of neomycin on calcium and polyphosphoinositide metabolism of guinea pig synaptosomes. *J. Neurochem.* 34: 750–752, 1980.
  115. HAMILL, O. P., A. MARTY, E. NEHER, B. SAKMANN, AND F. J. SIGWORTH. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* 391: 85–100, 1981.
  116. HATT, H., AND B. W. ACHE. Cyclic nucleotide- and inositol phosphate-gated ion channels in lobster olfactory receptor neurons. *Proc. Natl. Acad. Sci. USA* 91: 6264–6268, 1994.
  117. HEDLUND, B., AND G. M. SHEPHERD. Biochemical studies of muscarine receptors in the salamander olfactory epithelium. *FEBS Lett.* 162: 428–431, 1983.
  118. HELMCHEN, F., K. IMOTO, AND B. SAKMANN.  $\text{Ca}^{2+}$  buffering and action potential-evoked  $\text{Ca}^{2+}$  signaling in dendrites of pyramidal neurons. *Biophys. J.* 70: 1069–1081, 1996.
  119. HERVÉ, D., M. ROGARD, AND M. LÉVI-STRAUSS. Molecular analysis of the multiple  $G_{\text{olf}} \alpha$  subunit mRNAs in the rat brain. *Mol. Brain Res.* 32: 125–134, 1995.
  120. HILLE, B. *Ionic Channels of Excitable Membranes*. Sunderland, MA: Sinauer, 1992.
  121. HIRABAYASHI, S., AND N. SUZUKI. Co-existence of cAMP- and  $\text{IP}_3$  pathways in individual olfactory receptor cells in the rat. *Jpn. J. Tas. Sm. Res.* 1: 370–372, 1994.
  122. HIRONO, J., T. SATO, M. TONOIKE, AND M. TAKEBAYASHI. Local distribution of odor responsiveness of mouse olfactory receptor neurons. *Neurosci. Lett.* 174: 201–204, 1994.
  123. HONDA, E., J. H. TEETER, AND D. RESTREPO.  $\text{IP}_3$ -gated ion channels in rat olfactory cilia membrane. *Brain Res.* 703: 79–85, 1995.
  124. HOPKINS, A. E. The olfactory receptors in vertebrates. *J. Comp. Neurol.* 41: 253–289, 1926.
  125. HORN, R., AND A. MARTY. Muscarinic activation of ionic currents measured by a new whole-cell recording method. *J. Gen. Physiol.* 92: 145–159, 1988.
  126. HUGHES, B. P., A. M. AULD, AND G. J. BARRITT. Evidence that neomycin inhibits plasma membrane  $\text{Ca}^{2+}$  inflow in isolated hepatocytes. *Biochem. Pharmacol.* 37: 1357–1361, 1988.
  127. HUQUE, T., AND R. C. BRUCH. Odorant- and guanine nucleotide-stimulated phosphoinositide turnover in olfactory cilia. *Biochem. Biophys. Res. Commun.* 137: 36–42, 1986.
  128. ICHIKAWA, M., AND K. UEDA. Fine structure of the olfactory epithelium in the goldfish, *Carassius auratus*. *Cell Tissue Res.* 183: 445–455, 1977.
  129. INGI, T., AND G. V. RONNETT. Direct demonstration of a physiological role for carbon monoxide in olfactory receptor neurons. *J. Neurosci.* 15: 8214–8222, 1995.
  130. IVANOVA, T. T., AND J. CAPRIO. Odorant receptors activated by amino acids in sensory neurons of the channel catfish *Ictalurus punctatus*. *J. Gen. Physiol.* 102: 1085–1105, 1993.
  131. JIA, C., AND M. HALPERN. Subclasses of vomeronasal receptor neurons: differential expression of G proteins ( $G_{\text{ia2}}$  and  $G_{\text{oa}}$ ) and segregated projections to the accessory olfactory bulb. *Brain Res.* 719: 117–128, 1996.
  132. JONES, D. T., AND R. R. REED.  $G_{\text{olf}}$ : an olfactory neuron specific-G protein involved in odorant signal transduction. *Science* 244: 790–795, 1989.
  133. JORQUERA, O., R. LATORRE, AND P. LABARCA. Ion channel classes in purified olfactory cilia membranes: planar lipid bilayer studies. *Am. J. Physiol.* 269 (Cell Physiol. 38): C1235–C1244, 1995.
  134. JOSHI, H., M. L. GETCHELL, B. ZIELINSKI, AND T. V. GETCHELL. Spectrophotometric determination of cation concentrations in olfactory mucus. *Neurosci. Lett.* 82: 321–326, 1987.
  135. JULIFS, D. M., H.-J. FÜLLE, A. Z. ZHAO, M. D. HOUSLAY, D. L. GARBERS, AND J. A. BEAVO. A subset of olfactory neurons that selectively express cGMP-stimulated phosphodiesterase ( $\text{PDE}_2$ )



- and guanylyl cyclase-D define a unique olfactory signal transduction pathway. *Proc. Natl. Acad. Sci. USA* 94: 3388–3395, 1997.
136. JUNG, A., F. W. LISCHKA, J. ENGEL, AND D. SCHILD. Sodium/calcium exchanger in olfactory receptor neurones of *Xenopus laevis*. *Neuroreport* 5: 1741–1744, 1994.
  137. KAISLING, K.-E. Chemo-electrical transduction in insect olfactory receptors. *Annu. Rev. Neurosci.* 9: 121–145, 1986.
  138. KAISLING, K.-E. Single unit and electroantennogram recordings in insect olfactory organs. In: *Experimental Cell Biology of Taste and Olfaction. Current Techniques and Protocols*, edited by A. I. Spielman and J. G. Brand. Boca Raton, FL: CRC, 1995, p. 361–377.
  139. KAISLING, K.-E. Peripheral mechanisms of pheromone reception in moths. *Chem. Senses* 21: 257–268, 1996.
  140. KAISLING, K.-E., AND I. BOEKHOFF. Transduction and intracellular messengers in pheromone receptor cell of the moth *Antheraea polyphemus*. In: *Sensory Systems of Anthropods*, edited by K. Wiese, F. G. Gribakin, and A. V. Popov. Basel: Birkhäuser, 1993, p. 489–502.
  141. KALINOSKI, D. L., S. B. ALDINGER, A. G. BOYLE, T. HUQUE, J. F. MARECEK, G. D. PRESTWICH, AND D. RESTREPO. Characterization of a novel inositol 1,4,5-trisphosphate receptor in isolated olfactory cilia. *Biochem. J.* 281: 449–456, 1992.
  142. KANG, J., AND J. CAPRIO. In vivo responses of single olfactory receptor neurons in the channel catfish, *Ictalurus punctatus*. *J. Neurophysiol.* 73: 172–177, 1995.
  143. KASAI, H. Cytosolic Ca<sup>2+</sup> gradients, Ca<sup>2+</sup> binding proteins and synaptic plasticity. *Neurosci.* 16: 1–7, 1993.
  144. KASAI, H., AND O. H. PETERSEN. Spatial dynamics of second messengers: IP<sub>3</sub> and cAMP as long-range and associative messengers. *Trends Neurosci.* 17: 95–101, 1994.
  145. KASHIWAYANAGI, M. Dialysis of inositol 1,4,5-trisphosphate induces inward currents and Ca<sup>2+</sup> uptake in frog olfactory receptor cells. *Biochem. Biophys. Res. Commun.* 225: 666–671, 1996.
  146. KASHIWAYANAGI, M., AND K. KURIHARA. Odor discrimination in single turtle olfactory receptor neuron. *Neurosci. Lett.* 170: 233–236, 1994.
  147. KAUER, J. S. Olfactory receptor cell staining using horseradish. *Anat. Rec.* 200: 331–336, 1981.
  148. KAUER, J. S. Surface morphology of olfactory receptors. *J. Submicrosc. Cytol.* 15: 167–171, 1983.
  149. KAUER, J. S. Contributions of topography and parallel processing to odor coding in the vertebrate olfactory pathway. *Trends Neurosci.* 14: 79–85, 1991.
  150. KAUER, J. S. Video-rate imaging of potentiometric dyes in olfactory research. In: *Experimental Cell Biology of Taste and Olfaction. Current Techniques and Protocols*, edited by A. I. Spielman and J. G. Brand. Boca Raton, FL: CRC, 1995, p. 399–406.
  151. KAUPP, U. B. The cyclic nucleotide-gated channels of vertebrate photoreceptors and olfactory epithelium. *Trends Neurosci.* 14: 150–157, 1991.
  152. KAUPP, U. B. Family of cyclic nucleotide gated ion channels. *Curr. Opin. Neurobiol.* 5: 434–442, 1995.
  153. KAWAI, F., T. KURAHASHI, AND A. KANEKO. T-type Ca<sup>2+</sup> channel lowers the threshold of spike generation in the newt olfactory receptor cell. *J. Gen. Physiol.* 108: 525–535, 1996.
  154. KAWAI, F., T. KURAHASHI, AND A. KANEKO. Nonselective suppression of voltage-gated currents by odorants in the newt olfactory receptor cells. *J. Gen. Physiol.* 109: 265–272, 1997.
  155. KENT, P. F., M. M. MOZELL, S. J. MURPHY, AND D. E. HORNUNG. The interaction of imposed and inherent olfactory mucosal activity patterns and their composite representation in a mammalian species using voltage-sensitive dyes. *J. Neurosci.* 16: 345–353, 1996.
  156. KENT, P. F., S. L. YOUNGENTOB, AND P. R. SHEEHE. Odorant-specific spatial patterns in mucosal activity predict perceptual differences among odorants. *J. Neurophysiol.* 74: 1777–1781, 1995.
  157. KERN, R. C., T. P. KERR, AND T. V. GETCHELL. Ultrastructural localization of Na<sup>+</sup>/K<sup>+</sup>-ATPase in rodent olfactory epithelium. *Brain Res.* 546: 8–17, 1991.
  158. KERSCHBAUM, H. H., AND A. HERMANN. Calcium-binding proteins in chemoreceptors of *Xenopus laevis*. *Tissue Cell* 24: 719–724, 1992.
  159. KLEENE, S. J. Basal conductance of frog olfactory cilia. *Pflügers Arch.* 421: 374–380, 1992.
  160. KLEENE, S. J. Origin of the chloride current in olfactory transduction. *Neuron* 11: 123–132, 1993.
  161. KLEENE, S. J. The cyclic nucleotide-activated conductance in olfactory cilia: effects of Mg<sup>2+</sup> and Ca<sup>2+</sup>. *J. Membr. Biol.* 131: 237–243, 1993.
  162. KLEENE, S. J. Inhibition of olfactory cyclic nucleotide-activated current by calmodulin antagonists. *Br. J. Pharmacol.* 111: 469–472, 1994.
  163. KLEENE, S. J. Block by external calcium and magnesium of the cyclic-nucleotide-activated current in olfactory cilia. *Neuroscience* 66: 1001–1008, 1995.
  164. KLEENE, S. J., AND R. C. GESTELAND. Transmembrane currents in frog olfactory cilia. *J. Membr. Biol.* 120: 75–81, 1991.
  165. KLEENE, S. J., AND R. C. GESTELAND. Calcium-activated chloride conductance in frog olfactory cilia. *J. Neurosci.* 11: 3624–3629, 1991.
  166. KLEENE, S. J., R. C. GESTELAND, AND S. H. BRYANT. An electrophysiological survey of frog olfactory cilia. *J. Exp. Biol.* 195: 307–328, 1994.
  167. KLEENE, S. J., AND R. Y. K. PUN. Persistence of the olfactory receptor current in a wide variety of extracellular environments. *J. Neurophysiol.* 75: 1386–1391, 1996.
  168. KLEINEKE, J., C. DÜLS, AND H.-D. SÖLING. Subcellular compartmentation of guanine nucleotides and functional relationships between the adenine and guanine nucleotide systems in isolated hepatocytes. *FEBS Lett.* 107: 198–202, 1979.
  169. KOLESNIKOV, S. S., AND A. V. KOSOLAPOV. Cyclic nucleotide-activated channels in carp olfactory receptor cells. *Biochim. Biophys. Acta* 1150: 63–72, 1993.
  170. KOLESNIKOV, S. S., A. B. ZHAINAZAROV, AND A. V. KOSOLAPOV. Cyclic nucleotide-activated channels in the frog olfactory receptor plasma membrane. *FEBS Lett.* 266: 96–98, 1990.
  171. KOLNBERGER, I. Vergleichende Untersuchungen am Riechepithel, insbesondere des Jacobsonschen Organs von Amphibien, Reptilien und Säugetieren. *Z. Zellforsch. Mikrosk. Anat.* 122: 53–67, 1971.
  172. KOMATSU, H., I. MORI, J. RHEE, N. AKAIKE, AND Y. OHSHIMA. Mutations in a cyclic nucleotide-gated channel lead to abnormal thermosensation and chemosensation in *C. elegans*. *Neuron* 17: 707–718, 1996.
  173. KRAMER, R. H., AND S. A. SIEGELBAUM. Intracellular Ca<sup>2+</sup> regulates the sensitivity of cyclic nucleotide-gated channels in olfactory receptor neurons. *Neuron* 9: 897–906, 1992.
  174. KULKARNI, A. P., T. V. GETCHELL, AND M. L. GETCHELL. Neuronal nitric oxide synthase is localized in extrinsic nerves regulating perireceptor processes in the chemosensory nasal mucosae of rats and humans. *J. Comp. Neurol.* 345: 125–138, 1994.
  175. KURAHASHI, T. Activation by odorants of cation-selective conductance in the olfactory receptor cell isolated from the newt. *J. Physiol. (Lond.)* 419: 177–192, 1989.
  176. KURAHASHI, T. The response induced by intracellular cyclic AMP in isolated olfactory receptor cells of the newt. *J. Physiol. (Lond.)* 430: 355–371, 1990.
  177. KURAHASHI, T., AND A. KANEKO. High density cAMP-gated channels at the ciliary membrane in the olfactory receptor cell. *Neuroreport* 2: 5–8, 1991.
  178. KURAHASHI, T., AND A. KANEKO. Gating properties of the cAMP-gated channel in toad olfactory receptor cells. *J. Physiol. (Lond.)* 466: 287–302, 1993.
  179. KURAHASHI, T., A. KANEKO, AND T. SHIBUYA. Ionic mechanisms of the olfactory transduction studied on isolated receptor cells of the newt. *Neurosci. Res. Suppl.* 12: S85–S96, 1990.
  180. KURAHASHI, T., G. LOWE, AND G. H. GOLD. Suppression of odorant responses by odorants in olfactory receptor cells. *Science* 265: 118–120, 1994.
  181. KURAHASHI, T., AND A. MENINI. Mechanism of odorant adaptation in the olfactory receptor cell. *Nature* 385: 725–729, 1997.
  182. KURAHASHI, T., AND T. SHIBUYA. Membrane responses and permeability changes to odorants in the solitary olfactory receptor cells of newt. *Zool. Sci.* 6: 19–30, 1989.
  183. KURAHASHI, T., AND T. SHIBUYA. Ca<sup>2+</sup>-dependent adaptive properties in the solitary olfactory receptor cell of the newt. *Brain Res.* 515: 261–268, 1990.
  184. KURAHASHI, T., AND K.-W. YAU. Co-existence of cationic and chlo-

- ride components in odorant-induced current of vertebrate olfactory receptor cells. *Nature* 363: 71–74, 1993.
185. KURAHASHI, T., AND K.-W. YAU. Olfactory transduction: tale of an unusual chloride current. *Curr. Biol.* 4: 256–258, 1994.
  186. LABARCA, P., S. A. SIMON, AND R. R. ANHOLT. Activation by odorants of a multistate cation channel from olfactory cilia. *Proc. Natl. Acad. Sci. USA* 85: 944–947, 1988.
  187. LAMBERT, N., AND L. GROVER. The mechanism of biphasic GABA responses. *Science* 269: 928–929, 1995.
  188. LANCET, D. Vertebrate olfactory reception. *Annu. Rev. Neurosci.* 9: 329–355, 1986.
  189. LANCET, D., AND N. BEN-ARIE. Olfactory receptors. *Curr. Biol.* 3: 668–674, 1993.
  190. LATORRE, R., A. OBERHAUSER, P. LABARCA, AND O. ALVAREZ. Varieties of calcium-activated potassium channels. *Annu. Rev. Physiol.* 51: 385–399, 1989.
  191. LEIBOVICI, M., F. LAPOINTE, P. ALETTA, AND C. AYER-LE LIÈVRE. Avian olfactory receptors: differentiation of olfactory neurons under normal and experimental conditions. *Dev. Biol.* 175: 118–131, 1996.
  192. LEINDERS-ZUFALL, T., M. N. RAND, G. M. SHEPHERD, C. A. GREER, AND F. ZUFALL. Calcium entry through cyclic nucleotide-gated channels in individual cilia of olfactory receptor cells: spatio-temporal dynamics. *J. Neurosci.* 17: 4136–4148, 1997.
  193. LEINDERS-ZUFALL, T., G. M. SHEPHERD, AND F. ZUFALL. Regulation of cyclic nucleotide-gated channels and membrane excitability in olfactory receptor cells by carbon monoxide. *J. Neurophysiol.* 74: 1498–1508, 1995.
  194. LEINDERS-ZUFALL, T., G. M. SHEPHERD, AND F. ZUFALL. Modulation by cyclic GMP of the odour sensitivity of vertebrate olfactory receptor cells. *Proc. R. Soc. Lond. B Biol. Sci.* 236: 803–811, 1996.
  195. LEINDERS-ZUFALL, T., AND F. ZUFALL. Block of cyclic nucleotide-gated channels in salamander olfactory receptor neurons by the guanylyl cyclase inhibitor LY83583. *J. Neurophysiol.* 74: 2759–2762, 1995.
  196. LEVITAN, I. B. A family of calcium-dependent potassium channels from rat brain. *Neuron* 2: 1031–1041, 1989.
  197. LIMAN, E. R. Pheromone transduction in the vomeronasal organ. *Curr. Opin. Neurobiol.* 6: 487–493, 1996.
  199. LIMAN, E. R., AND L. B. BUCK. A second subunit of the olfactory cyclic nucleotide-gated channel confers high sensitivity to cAMP. *Neuron* 13: 611–621, 1994.
  200. LIMAN, E. R., AND D. P. COREY. Electrophysiological characterization of chemosensory neurons from the mouse vomeronasal organ. *J. Neurosci.* 79: 1606–1613, 1997.
  201. LINDEMANN, B. Taste reception. *Physiol. Rev.* 76: 719–766, 1996.
  202. LINGREL, J. B., AND T. KUNTZWEILER. Na<sup>+</sup>,K<sup>+</sup>-ATPase. *J. Biol. Chem.* 269: 19659–19662, 1994.
  203. LIPP, P., AND E. NIGGLI. Ratiometric confocal Ca<sup>2+</sup>-measurements with visible wavelength indicators in isolated cardiac myocytes. *Cell Calcium* 14: 359–372, 1993.
  204. LISCHKA, F. W., AND D. SCHILD. Standing calcium gradients in olfactory receptor neurons can be abolished by amiloride or Ruthenium Red. *J. Gen. Physiol.* 102: 817–831, 1993.
  205. LISCHKA, F. W., AND D. SCHILD. Effects of nitric oxide upon olfactory receptor neurones in *Xenopus laevis*. *Neuroreport* 4: 582–584, 1993.
  206. LIU, M., T.-Y. CHEN, B. AHAMED, J. LI, AND K.-W. YAU. Calcium-calmodulin modulation of the olfactory cyclic nucleotide-gated cation channel. *Science* 266: 1348–1354, 1994.
  207. LLODHI, S., N. D. WEINER, AND J. SCHACHT. Interactions of neomycin and calcium in synaptosomal membranes and phosphoinositide monolayers. *Biochim. Biophys. Acta* 426: 781–785, 1976.
  208. LO, Y. H., S. L. BELLIS, L.-J. CHENG, J. PANG, T. M. BRADLEY, AND D. E. RHOADS. Signal transduction for taurocholic acid in the olfactory system of Atlantic salmon. *Chem. Senses* 19: 371–380, 1994.
  209. LO, Y. H., T. M. BRADLEY, AND D. E. RHOADS. L-Alanine binding sites and Na<sup>+</sup>,K<sup>+</sup>-ATPase in cilia and other membrane fractions from olfactory rosettes of Atlantic salmon. *Comp. Biochem. Physiol. B Biochem.* 98: 121–126, 1991.
  210. LO, Y. H., T. M. BRADLEY, AND D. E. RHOADS. Stimulation of Ca<sup>2+</sup>-regulated olfactory phospholipase C by amino acids. *Biochemistry* 32: 12358–12362, 1993.
  211. LO, Y. H., T. M. BRADLEY, AND D. E. RHOADS. High-affinity Ca<sup>2+</sup>,Mg<sup>2+</sup>-ATPase in plasma membrane-rich preparations from olfactory epithelium of Atlantic salmon. *Biochim. Biophys. Acta* 1192: 153–158, 1994.
  212. LOWE, G., AND G. H. GOLD. The spatial distributions of odorant sensitivity and odorant-induced currents in salamander olfactory receptor cells. *J. Physiol. (Lond.)* 442: 147–168, 1991.
  213. LOWE, G., AND G. H. GOLD. Contribution of the ciliary cyclic nucleotide-gated conductance to olfactory transduction in the salamander. *J. Physiol. (Lond.)* 462: 175–196, 1993.
  214. LOWE, G., AND G. H. GOLD. Nonlinear amplification by calcium-dependent chloride channels in olfactory receptor cells. *Nature* 366: 283–286, 1993.
  215. LOWE, G., AND G. H. GOLD. Olfactory transduction is intrinsically noisy. *Proc. Natl. Acad. Sci. USA* 92: 7864–7868, 1995.
  216. LOWE, G., T. NAKAMURA, AND G. H. GOLD. Adenylate cyclase mediates transduction for a wide variety of odorants. *Proc. Natl. Acad. Sci. USA* 86: 5641–5645, 1989.
  217. LUCERO, M. T., F. T. HERRIGAN, AND W. F. GILLY. Electrical responses to chemical stimulation of squid olfactory receptor cells. *J. Exp. Biol.* 162: 231–249, 1992.
  218. LUDWIG, J., T. MARGALIT, E. EISMANN, D. LANCET, AND U. B. KAUPP. Primary structure of cAMP-gated channel from bovine olfactory epithelium. *FEBS Lett.* 270: 24–29, 1990.
  219. LYNCH, J. W., AND P. H. BARRY. Action potentials initiated by single channels opening in a small neuron (rat olfactory receptor). *Biophys. J.* 55: 755–768, 1989.
  220. LYNCH, J. W., AND P. H. BARRY. Slowly activating K<sup>+</sup> channels in rat olfactory receptor neurons. *Proc. R. Soc. Lond. B Biol. Sci.* 244: 219–225, 1991.
  221. LYNCH, J. W., AND P. H. BARRY. Properties of transient K<sup>+</sup> currents and underlying single K<sup>+</sup> channels in rat olfactory neurons. *J. Gen. Physiol.* 97: 1043–1072, 1991.
  222. LYNCH, J. W., AND P. H. BARRY. Inward rectification in rat olfactory receptor neurons. *Proc. R. Soc. Lond. B Biol. Sci.* 243: 149–153, 1991.
  223. LYNCH, J. W., AND B. LINDEMANN. Cyclic nucleotide-gated channels of the rat olfactory receptor cells: divalent cations control the sensitivity to cAMP. *J. Gen. Physiol.* 103: 87–106, 1994.
  224. MAIDA, R., G. ZIEGELBERGER, AND K.-E. KAISLING. Esterase activity in the olfactory sensilla of the silkworm *Antheraea polyphemus*. *Neuroreport* 6: 822–824, 1995.
  225. MANIA-FARNELL, B. L., A. I. FARBMAN, AND R. C. BRUCH. Bromocriptine, a dopamine D<sub>2</sub> receptor agonist, inhibits adenylyl cyclase activity in rat olfactory epithelium. *Neuroscience* 57: 173–180, 1993.
  226. MASUKAWA, L. M., B. HEDLUND, AND G. M. SHEPHERD. Electrophysiological properties of identified cells in the in vitro olfactory epithelium of the tiger salamander. *J. Neurosci.* 5: 128–135, 1985.
  227. MAUE, R. A., AND V. E. DIONNE. Patch-clamp studies of isolated mouse olfactory receptor neurons. *J. Gen. Physiol.* 90: 95–125, 1987.
  228. McCLINTOCK, T. S., AND B. W. ACHE. Ionic currents and ion channels of lobster olfactory receptor neurons. *J. Gen. Physiol.* 94: 1085–1099, 1989.
  229. McCLINTOCK, T. S., AND B. W. ACHE. Hyperpolarizing receptor potentials in lobster olfactory receptor cells: implications for transduction and mixture suppression. *Chem. Senses* 14: 637–647, 1989.
  230. MCCORMICK, D. A., AND H.-C. PAPE. Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. *J. Physiol. (Lond.)* 431: 291–318, 1990.
  231. MENCIO, B. P. M. Ciliated and microvillous structures of rat olfactory and nasal respiratory epithelia. *Cell Tissue Res.* 235: 225–241, 1984.
  232. MENCIO, B. P. M. Ultrastructural studies on membrane, cytoskeletal, mucous and protective compartments in olfaction. *Microsc. Res. Tech.* 22: 215–224, 1992.
  233. MENCIO, B. P. M. Freeze-fracture, deep-etch, and freeze-substitution studies of olfactory epithelia, with special emphasis on immunocytochemical variables. *Microsc. Res. Tech.* 32: 337–356, 1995.
  234. MENCIO, B. P. M., R. C. BRUCH, B. DAU, AND W. DANHO. Ultra-

- structural localization of olfactory transduction components: the G protein subunit  $G_{\text{olf}} \alpha$  and type III adenylyl cyclase. *Neuron* 8: 441–453, 1992.
235. MENCO, B. P. M., AND A. I. FARBMAN. Ultrastructural evidence for multiple mucous domains in frog olfactory epithelium. *Cell Tissue Res.* 270: 47–56, 1992.
236. MENCO, B. P. M., AND A. I. FARBMAN. Genesis of cilia and microvilli of rat nasal epithelia during pre-natal development. II. Olfactory epithelium, a morphometric analysis. *J. Cell Sci.* 78: 311–336, 1995.
237. MENCO, B. P. M., F. D. TEKULA, A. I. FARBMAN, AND W. DANHO. Developmental expression of G-proteins and adenylyl cyclase in peripheral olfactory systems. Light microscopic and freeze-substitution electron microscopic immunocytochemistry. *J. Neurocytol.* 23: 708–727, 1994.
238. MENINI, A., C. PICCO, AND S. FIRESTEIN. Quantal-like current fluctuations induced by odorants in olfactory receptor cells. *Nature* 373: 435–437, 1995.
239. MEYER, D. L., A. G. JADHAO, AND E. KICLITER. Soybean agglutinin binding by primary olfactory and primary accessory olfactory projections in different frogs. *Brain Res.* 722: 222–226, 1996.
240. MICHEL, W. C., AND B. W. ACHE. Cyclic nucleotides mediate an odor-evoked potassium conductance in lobster olfactory receptor cells. *J. Neurosci.* 12: 3979–3984, 1992.
- 240a. MICHEL, W. C., AND B. W. ACHE. Odor-evoked inhibition in primary olfactory receptor neurons. *Chem. Senses* 19: 11–24, 1994.
241. MICHEL, W. C., T. S. McCLINTOCK, AND B. W. ACHE. Inhibition of lobster olfactory receptor cells by an odor-activated potassium conductance. *J. Neurophysiol.* 65: 446–453, 1991.
242. MILLER, M. L., A. ANDRINGA, J. E. EVANS, AND L. HASTINGS. Microvillar cells of the olfactory epithelium: morphology and regeneration following exposure to toxic compounds. *Brain Res.* 669: 1–9, 1995.
243. MINOR, A. V., K. A. BYKOV, A. V. DMITRIEV, AND S. N. SKACHKOV. Potassium, calcium, sodium and chloride concentrations in olfactory mucus measured by means of ion-selective microelectrodes. *Sensory System* 4: 220–227, 1990.
244. MINOR, A. V., K. A. BYKOV, A. V. DMITRIEV, AND S. N. SKACHKOV. Extracellular ion concentrations in the olfactory epithelium: steady state and changes during excitation (Abstract). *Chem. Senses* 17: 864, 1992.
245. MIYAMOTO, T., D. RESTREPO, E. J. CRAGOE, JR., AND J. H. TEETER.  $IP_3$ - and cAMP-induced responses in isolated olfactory receptor neurons from the channel catfish. *J. Membr. Biol.* 127: 173–183, 1992.
246. MIYAMOTO, T., D. RESTREPO, AND J. H. TEETER. Voltage-dependent and odorant-regulated currents in isolated olfactory receptor neurons of the channel catfish. *J. Gen. Physiol.* 99: 505–529, 1992.
247. MOMBARTS, P. Targeting olfaction. *Curr. Opin. Neurobiol.* 6: 481–486, 1996.
248. MORALES, B., P. LABARCA, AND J. BACIGALUPO. A ciliary  $K^+$  conductance sensitive to charybdotoxin underlies inhibitory responses in toad olfactory receptor neurons. *FEBS Lett.* 359: 41–44, 1995.
249. MORALES, B., G. UGARTE, P. LABARCA, AND J. BACIGALUPO. Inhibitory  $K^+$  current activated by odorant in toad olfactory neurons. *Proc. R. Soc. Lond. B Biol. Sci.* 257: 235–242, 1994.
250. MORAN, D. T., J. C. ROWLEY, III, G. R. ALKEN, AND B. W. JAFEK. Ultrastructural neurobiology of the olfactory mucosa of the brown trout, *Salmo trutta*. *Microsc. Res. Tech.* 23: 28–48, 1992.
251. MORAN, D. T., J. C. ROWLEY, III, B. W. JAFEK, AND M. A. LOVELL. The fine structure of the olfactory mucosa in man. *J. Neurocytol.* 11: 721–746, 1982.
252. MORI, K., AND Y. YOSHIHARA. Molecular recognition and olfactory processing in the mammalian olfactory system. *Prog. Neurobiol.* 45: 585–619, 1995.
253. MORRISON, E. E., AND R. M. COSTANZO. Morphology of the human olfactory epithelium. *J. Comp. Neurol.* 297: 1–13, 1990.
254. MULLER, J. F., AND R. E. MARC. Three distinct morphological classes of receptors in fish olfactory organs. *J. Comp. Neurol.* 222: 482–495, 1984.
255. NAKAMURA, T., AND G. H. GOLD. A cyclic nucleotide-gated conductance in olfactory receptor cilia. *Nature* 325: 442–444, 1987.
256. NAKAMURA, T., H.-H. LEE, H. KOBAYASHI, AND T.-O. SATOH. Gated conductances in native and reconstituted membranes from frog olfactory cilia. *Biophys. J.* 70: 813–817, 1996.
257. NAKAMURA, T., K. TSURU, AND S. MIYAMOTO. Regulation of  $Ca^{2+}$  concentration by second messengers in newt olfactory receptor cell. *Neurosci. Lett.* 171: 197–200, 1994.
258. NEF, S., I. ALLAMAN, H. FIUMELLI, E. DE CASTRO, AND P. NEF. Olfaction in birds: differential embryonic expression of nine putative odorant receptor genes in the avian olfactory system. *Mech. Dev.* 55: 65–77, 1996.
259. NEHER, E., AND G. J. AUGUSTINE. Calcium gradients and buffers in bovine chromaffin cells. *J. Physiol. (Lond.)* 450: 273–301, 1992.
260. NEVITT, G. A., AND W. J. MOODY. An electrophysiological characterization of ciliated olfactory receptor cells of the coho salmon *Oncorhynchus kisutch*. *J. Exp. Biol.* 166: 1–17, 1992.
261. NGAI, J., A. CHESS, M. M. DOWLING, N. NECLES, E. R. MACAGNO, AND R. AXEL. Coding of olfactory information: topography of odorant receptor expression in the catfish olfactory epithelium. *Cell* 72: 667–680, 1993.
262. NGAI, J., M. M. DOWLING, L. BUCK, R. AXEL, AND A. CHESS. The family of genes encoding odorant receptors in the channel catfish. *Cell* 72: 657–666, 1993.
263. NICKELL, W. T., A. B. NORMAN, L. M. WYATT, AND M. T. SHIPLEY. Olfactory bulb DA receptors may be located on terminals of the olfactory nerve. *Neuroreport* 2: 9–12, 1991.
264. OKADA, Y., J. H. TEETER, AND D. RESTREPO. Inositol 1,4,5-trisphosphate-gated conductance in isolated rat olfactory neurons. *J. Neurophysiol.* 71: 595–602, 1994.
265. OKAMOTO, K., Y. TOKUMITSU, AND M. KASHIWAYANAGI. Adenylyl cyclase activity in turtle vomeronasal and olfactory epithelium. *Biochem. Biophys. Res. Commun.* 220: 98–101, 1996.
266. OLEMPSKA-BEER, Z., AND E. BAUTZ-FREEZE. Optimal extraction conditions for high-performance liquid chromatographic determination of nucleotides in yeast. *Anal. Biochem.* 140: 236–245, 1984.
267. PACE, U., E. HANSKI, Y. SALOMON, AND D. LANCET. Odorant-sensitive adenylyl cyclase may mediate olfactory reception. *Nature* 316: 255–258, 1985.
268. PACE, U., AND D. LANCET. Olfactory GTP-binding protein: signal-transducing polypeptide of vertebrate chemosensory neurons. *Proc. Natl. Acad. Sci. USA* 83: 4947–4951, 1986.
269. PANG, J., Y. H. LO, J. M. CHANDLEE, AND D. E. RHOADS. A subtype of the metabotropic glutamate receptor family in the olfactory system of Atlantic salmon. *FEBS Lett.* 354: 301–304, 1994.
270. PELOSI, P. Odorant-binding proteins. *Crit. Rev. Biochem. Mol. Biol.* 29: 199–228, 1994.
271. PELOSI, P. Perireceptor events in olfaction. *J. Neurobiol.* 30: 3–19, 1996.
272. PERSAUD, K. C., J. A. DESIMONE, M. L. GETCHELL, G. L. HECK, AND T. V. GETCHELL. Ion transport across olfactory mucosa: the basal and odorant-stimulated states. *Biochim. Biophys. Acta* 902: 65–79, 1987.
273. PERSAUD, K. C., G. L. HECK, S. K. DESIMONE, T. V. GETCHELL, AND J. A. DESIMONE. Ion transport across the frog olfactory mucosa: the action of cyclic nucleotides on the basal and odorant-stimulated states. *Biochim. Biophys. Acta* 944: 49–62, 1988.
274. PFEUFFER, E., S. MOLLNER, D. LANCET, AND T. PFEUFFER. Olfactory adenylyl cyclase. Identification and purification of a novel enzyme form. *J. Biol. Chem.* 264: 18803–18807, 1989.
275. PIXLEY, S. K., AND R. Y. K. PUN. Cultured rat olfactory neurons are excitable and respond to odors. *Dev. Brain Res.* 53: 125–130, 1990.
276. PONGRACZ, F., S. FIRESTEIN, AND G. M. SHEPHERD. Electrotone structure of olfactory sensory neurons analyzed by intracellular and whole cell patch techniques. *J. Neurophysiol.* 65: 747–758, 1991.
277. PUN, R. Y. K., S. J. KLEENE, AND R. C. GESTELAND. Guanine nucleotides modulate steady-state inactivation of voltage-gated sodium channels in frog olfactory receptor neurons. *J. Membr. Biol.* 142: 103–111, 1994.
278. PUSCH, M., AND E. NEHER. Rates of diffusional exchange between small cells and a measuring patch pipette. *Pflügers Arch.* 411: 204–211, 1988.
279. RAFOLS, J. A., AND T. V. GETCHELL. Morphological relations between the receptor cells, sustentacular cells, and Schwann cells in

- the olfactory mucosa of the salamander. *Anat. Rec.* 206: 87–101, 1983.
280. RAJENDRA, S., J. W. LYNCH, AND P. H. BARRY. An analysis of Na<sup>+</sup> currents in rat olfactory receptor neurons. *Pflügers Arch.* 420: 342–346, 1992.
  281. RAMING, K., J. KRIEGER, J. STROTMANN, I. BOEKHOFF, S. KUBICK, C. BAUMSTARK, AND H. BREER. Cloning and expression of odorant receptors. *Nature* 361: 353–356, 1993.
  282. RAWSON, N. E., G. GOMEZ, B. J. COWART, J. G. BRAND, L. D. LOWRY, E. A. PRIBITKIN, AND D. RESTREPO. Selectivity and response characteristics of human olfactory neurons. *J. Neurophysiol.* 77: 1606–1613, 1997.
  283. REED, R. R. Signaling pathways in odorant detection. *Neuron* 8: 205–209, 1992.
  284. REESE, T. S. Olfactory cilia in the frog. *J. Cell Biol.* 25: 209–230, 1965.
  285. RESSLER, K. J., S. L. SULLIVAN, AND L. B. BUCK. A zonal organization of odorant receptor gene expression in the olfactory epithelium. *Cell* 73: 597–609, 1993.
  286. RESSLER, K. J., S. L. SULLIVAN, AND L. B. BUCK. Information coding in the olfactory system: evidence for a stereotyped and highly organized epitope map in the olfactory bulb. *Cell* 79: 1245–1255, 1994.
  287. RESTREPO, D., I. BOEKHOFF, AND H. BREER. Rapid kinetic measurements of second messenger formation in olfactory cilia from channel catfish. *Am. J. Physiol.* 264 (*Cell Physiol.* 33): C906–C911, 1993.
  288. RESTREPO, D., AND A. G. BOYLE. Stimulation of olfactory receptors alters regulation of [Ca<sub>i</sub>] in olfactory neurons of the catfish (*Ictalurus punctatus*). *J. Membr. Biol.* 120: 223–232, 1991.
  289. RESTREPO, D., D. J. KOZODY, L. J. SPINELLI, AND P. A. KNAUF. pH homeostasis in promyelocytic leukemic HL60 cells. *J. Gen. Physiol.* 92: 489–507, 1988.
  290. RESTREPO, D., T. MIYAMOTO, B. P. BRYANT, AND J. H. TEETER. Odor stimuli trigger influx of calcium into olfactory neurons of the channel catfish. *Science* 249: 1166–1168, 1990.
  291. RESTREPO, D., Y. OKADA, AND J. H. TEETER. Odorant-regulated Ca<sup>2+</sup> gradients in rat olfactory neurons. *J. Gen. Physiol.* 102: 907–924, 1993.
  292. RESTREPO, D., Y. OKADA, J. H. TEETER, L. D. LOWRY, AND B. COWART. Human olfactory neurons respond to odor stimuli with an increase in cytoplasmic Ca<sup>2+</sup>. *Biophys. J.* 64: 1961–1966, 1993.
  293. RESTREPO, D., AND J. H. TEETER. Olfactory neurons exhibit heterogeneity in depolarization-induced calcium changes. *Am. J. Physiol.* 258 (*Cell Physiol.* 27): C1051–C1061, 1990.
  294. RESTREPO, D., J. H. TEETER, E. HONDA, A. G. BOYLE, J. F. MARCEK, G. D. PRESTWICH, AND D. L. KALINOSKI. Evidence for an InsP<sub>3</sub>-gated channel protein in isolated rat olfactory cilia. *Am. J. Physiol.* 263 (*Cell Physiol.* 32): C667–C673, 1992.
  295. RESTREPO, D., J. H. TEETER, AND D. SCHILD. Second messenger signaling in olfactory transduction. *J. Neurobiol.* 30: 37–48, 1996.
  296. RESTREPO, D., M. M. ZVIMAN, AND N. E. RAWSON. Imaging of intracellular calcium in chemosensory receptor cells. In: *Experimental Cell Biology of Taste and Olfaction*, edited by A. I. Spielman and J. G. Brand. Boca Raton, FL: CRC, 1995, p. 387–398.
  297. RONNETT, G. V., H. CHO, L. D. HESTER, S. F. WOOD, AND S. H. SNYDER. Odorants differentially enhance phosphoinositide turnover and adenylyl cyclase in olfactory receptor neuronal cultures. *J. Neurosci.* 13: 1751–1758, 1993.
  298. RONNETT, G. V., AND S. H. SNYDER. Molecular messengers of olfaction. *Trends Neurosci.* 15: 508–513, 1992.
  299. ROSKAMS, A. J., D. S. BREDT, T. M. DAWSON, AND G. V. RONNETT. Nitric oxide mediates the formation of synaptic connections in developing and regenerating olfactory receptor neurons. *Neuron* 13: 289–299, 1994.
  300. ROWLEY, J. C. I., D. T. MORAN, AND B. W. JAFEK. Peroxidase backfills suggest the mammalian olfactory epithelium contains a second morphologically distinct class of bipolar sensory neuron: the microvillar cell. *Brain Res.* 502: 387–400, 1989.
  301. SAKMANN, B., AND E. NEHER. Geometric parameters of pipettes and membrane patches. In: *Single Channel Recording*, edited by B. Sakmann and E. Neher. New York: Plenum, 1995, p. 637–650.
  302. SATO, T., J. HIRONO, M. TONOIKE, AND M. TAKEBAYASHI. Two types of increases in free Ca<sup>2+</sup> evoked by odor in isolated frog olfactory receptor neurons. *Neuroreport* 2: 229–232, 1991.
  303. SATO, T., J. HIRONO, M. TONOIKE, AND M. TAKEBAYASHI. Tuning specificities to aliphatic odorants in mouse olfactory receptor neurons and their local distribution. *J. Neurophysiol.* 72: 2980–2989, 1994.
  304. SCHILD, D. Principles of odor coding and a neural network for odor discrimination. *Biophys. J.* 54: 1001–1011, 1988.
  305. SCHILD, D. Whole-cell currents in olfactory receptor cells of *Xenopus laevis*. *Exp. Brain Res.* 78: 223–232, 1989.
  306. SCHILD, D. Laser scanning microscopy and calcium imaging. *Cell Calcium* 19: 281–296, 1996.
  307. SCHILD, D., AND J. BISCHOFBERGER. Ca<sup>2+</sup> modulates an unspecific cation conductance in olfactory cilia of *Xenopus laevis*. *Exp. Brain Res.* 84: 187–194, 1991.
  308. SCHILD, D., I. BOEKHOFF, F. W. LISCHKA, AND H. BREER. Signal transduction in olfactory neurons of *Xenopus laevis*. In: *Proceedings of the 20th Goettingen Neurobiology Conference*, edited by N. Elsner and D. W. Richter. Stuttgart, Germany: Thieme, 1992.
  309. SCHILD, D., A. JUNG, AND H. A. SCHULTENS. Localization of calcium entry through calcium channels in olfactory receptor neurons using a laser scanning microscope and the calcium indicator dyes Fluo-3 and Fura-Red. *Cell Calcium* 15: 341–348, 1994.
  310. SCHILD, D., AND F. W. LISCHKA. Amiloride-insensitive cation conductance in *Xenopus laevis* olfactory neurons: a combined patch clamp and calcium imaging analysis. *Biophys. J.* 66: 299–304, 1994.
  311. SCHILD, D., F. W. LISCHKA, AND D. RESTREPO. InsP<sub>3</sub> causes an increase in apical [Ca<sup>2+</sup>]<sub>i</sub> by activating two distinct components in vertebrate olfactory receptor cells. *J. Neurophysiol.* 73: 862–866, 1995.
  312. SCHLEICHER, S., I. BOEKHOFF, J. ARRIZA, R. J. LEFKOWITZ, AND H. BREER. A β-adrenergic receptor kinase-like enzyme is involved in olfactory signal termination. *Proc. Natl. Acad. Sci. USA* 90: 1420–1424, 1993.
  313. SCHMIEDEL-JACOB, I., W. C. MICHEL, P. A. V. ANDERSON, AND B. W. ACHE. Whole cell recording from lobster olfactory receptor cells: multiple ionic bases for the receptor potential. *Chem. Senses* 15: 397–405, 1990.
  314. SCHULTZE, M. J. S. Über die Endigungsweise des Geruchsnerven und die Epithelialgebilde der Nasenschleimhaut. *Verhandl. Akad. Wissensch. Berlin* 21: 504–514, 1856.
  315. SCHULTZE, M. J. S. Untersuchungen über den Bau der Nasenschleimhaut, namentlich die Structur und Endigungsweise der Geruchsnerven bei dem Menschen und den Wirbeltieren. *Abhandl. d. Nat. Ges. zu Halle* 7: 1–99, 1863.
  316. SCOTT, J. W. Central processing of olfaction. *J. Steroid Biochem. Mol. Biol.* 39: 593–600, 1991.
  317. SEGALL, J. E., S. M. BLOCK, AND H. C. BERG. Temporal comparisons in bacterial chemotaxis. *Proc. Natl. Acad. Sci. USA* 83: 8987–8991, 1986.
  318. SEIFERT, K. Die Ultrastruktur des Riechepithels beim Makrosomatiker. Eine elektronenmikroskopische Untersuchung. In: *Normale und pathologische Anatomie. Monographien in zwangloser Folge*, edited by W. Bargmann and W. Doerr. Stuttgart, Germany: Thieme, 1970, p. 1–99.
  319. SEIFERT, K. Licht- und elektronenmikroskopische Untersuchungen am Jakobsonschen Organ (Organum vomeronasale) der Katze. *Arch. Klin. Exp. Ohren Nasen Kehlkopfheilkd.* 200: 233–251, 1971.
  320. SENGUPTA, P., J. H. CHOU, AND C. I. BARGMANN. Odr-10 encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. *Cell* 84: 899–909, 1996.
  321. SENGUPTA, P., H. A. COLBERT, AND C. I. BARGMANN. The *C. elegans* gene odr-7 encodes an olfactory-specific member of the nuclear receptor superfamily. *Cell* 79: 971–980, 1994.
  322. SHEPHERD, G. M. Discrimination of molecular signals by the olfactory receptor neuron. *Neuron* 13: 771–790, 1994.
  323. SHIPLEY, M. T., AND M. ENNIS. Functional organization of olfactory system. *J. Neurobiol.* 30: 123–176, 1996.
  324. SHIRLEY, S. G., C. J. ROBONSON, K. DICKINSON, R. AUJLA, AND G. H. DODD. Olfactory adenylyl cyclase of the rat. *Biochem. J.* 240: 605–607, 1986.
  325. SICARD, G., AND A. HOLLEY. Receptor cell responses to odorants:

- similarities and differences among odorants. *Brain Res.* 292: 283–296, 1984.
326. SKLAR, P. B., R. R. ANHOLT, AND S. H. SNYDER. The odorant-sensitive adenylate cyclase of olfactory receptor cells. Differential stimulation by distinct classes of odorants. *J. Biol. Chem.* 261: 15538–15543, 1986.
327. STENGL, M. Intracellular-messenger-mediated cation channels in cultured olfactory receptor neurons. *J. Exp. Biol.* 178: 125–147, 1993.
328. STENGL, M. Inositol-trisphosphate-dependent calcium currents precede cation currents in insect olfactory receptor neurons in vitro. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* 174: 187–194, 1994.
329. STENGL, M., H. HATT, AND H. BREER. Peripheral processes in insect olfaction. *Annu. Rev. Physiol.* 54: 665–681, 1992.
330. STRIGGOW, F., AND R. BOHNENSACK. Inositol 1,4,5-trisphosphate activates receptor-mediated calcium entry by two different pathways in hepatocytes. *Eur. J. Biochem.* 222: 229–234, 1994.
331. SUTTERLIN, A. M., AND N. SUTTERLIN. Electrical responses of the olfactory epithelium of Atlantic salmon (*Salmo salar*). *J. Fish. Res. Bd. Canada* 28: 565–572, 1971.
332. SUZUKI, N. Single cyclic nucleotide-activated ion channels in olfactory receptor cell soma membranes. *Neurosci. Res. Suppl.* 12, Suppl.: S113–S126, 1990.
333. SUZUKI, N. IP<sub>3</sub>-activated ion channel activities in olfactory receptor neurons from different vertebrate species. In: *Olfaction and Taste XI*, edited by K. Kurihara, N. Suzuki, and H. Ogawa. Tokyo: Springer-Verlag, 1994, p. 173–177.
334. SUZUKI, N., AND D. TUCKER. Amino acids as olfactory stimuli in freshwater catfish, *Ictalurus catus* (Linn.). *Comp. Biochem. Physiol.* 40: 399–404, 1971.
335. TANIGUCHI, M., M. KASHIWAYANAGI, AND K. KURIHARA. Intracellular injection of inositol 1,4,5-trisphosphate increases a conductance in membranes of turtle vomeronasal receptor neurons in the slice preparation. *Neurosci. Lett.* 188: 5–8, 1995.
336. TANIGUCHI, M., M. KASHIWAYANAGI, AND K. KURIHARA. Intracellular dialysis of cyclic nucleotides induces inward currents in turtle vomeronasal receptor neurons. *J. Neurosci.* 16: 1239–1246, 1996.
337. TARELLUS, E., J. NOE, AND H. BREER. Calcium signals in olfactory neurons. *Biochim. Biophys. Acta* 1269: 129–138, 1995.
338. THEISEN, B., E. ZEISKE, AND H. BREUCKER. Functional morphology of the olfactory organs in the spiny dogfish (*Squalus acanthias* L.) and the small-spotted catshark (*Scyliorhinus canicula* L.). *Acta Zool.* 67: 73–86, 1986.
339. TOKIMASA, T., AND T. AKASU. Cyclic AMP regulates an inward rectifying sodium-potassium current in dissociated bull-frog sympathetic neurones. *J. Physiol. (Lond.)* 420: 409–429, 1990.
340. TORRE, V., J. F. ASHMORE, T. D. LAMB, AND A. MENINI. Transduction and adaptation in sensory receptor cells. *J. Neurosci.* 15: 7757–7768, 1995.
341. TROEMEL, E. R., J. H. CHOU, N. D. DWYER, H. A. COLBERT, AND C. I. BARGMANN. Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. *Cell* 83: 207–218, 1995.
342. TROMBLEY, P. Q., AND G. L. WESTBROOK. Voltage-gated currents in identified rat olfactory receptor neurons. *J. Neurosci.* 11: 435–444, 1991.
343. TROTIER, D. A patch-clamp analysis of membrane currents in salamander olfactory receptor cells. *Pflügers Arch.* 407: 589–595, 1986.
344. TROTIER, D. Intensity coding in olfactory receptor cells. *Cell Biol.* 5: 47–54, 1994.
345. TROTIER, D., K. B. DOVING, AND J.-F. ROSIN. Voltage-dependent currents in microvillar receptor cells of the frog vomeronasal organ. *Eur. J. Neurosci.* 5: 995–1002, 1993.
346. VAN HOUTEN, J. Chemosensory transduction in eukaryotic organisms: trends for neuroscience? *Trends Neurosci.* 17: 62–71, 1994.
347. VANNELLI, G. B., F. ENSOLI, R. ZONEFRATI, Y. KUBOTA, A. ARCANGELI, A. BECCHETTI, G. CAMICI, T. BARNI, C. J. THIELE, AND G. C. BALBONI. Neuroblast long-term cell cultures from human fetal olfactory epithelium respond to odors. *J. Neurosci.* 15: 4382–4394, 1995.
348. VASSAR, R., S. K. CHAO, R. SITCHERAN, J. M. NUÑEZ, L. B. VOSS-HALL, AND R. AXEL. Topographic organization of sensory projections to the olfactory bulb. *Cell* 79: 981–991, 1994.
349. VASSAR, R., J. NGAI, AND R. AXEL. Spatial segregation of odorant receptor expression in the mammalian olfactory epithelium. *Cell* 74: 309–318, 1993.
350. VERMA, A., D. J. HIRSCH, C. E. GLATT, G. V. RONNETT, AND S. H. SNYDER. Carbon monoxide: a putative neural messenger. *Science* 259: 381–384, 1993.
351. VODYANOY, I., AND R. B. MURPHY. Single-channel fluctuations in biomolecular lipid membranes induced by rat olfactory epithelial homogenates. *Science* 220: 717–719, 1996.
352. WANG, R. T., AND M. HALPERN. Light and electron microscopic observations on the normal structure of the vomeronasal organ of garter snakes. *J. Morphol.* 164: 47–67, 1980.
353. WEYAND, I., M. GODDE, S. FRINGS, J. WEINER, F. MULLER, W. ALTENHOFEN, H. HATT, AND U. B. KAUPP. Cloning and functional expression of a cyclic-nucleotide-gated channel from mammalian sperm. *Nature* 368: 859–863, 1994.
354. WOOLZIN, B., T. SUNDERLAND, B.-B. ZHENG, J. RESAU, B. DUFY, J. BARKER, R. SWERDLOW, AND M. J. COON. Continuous culture of neuronal cells from adult human olfactory epithelium. *J. Mol. Neurosci.* 3: 137–146, 1996.
355. YANO, J., D. FRAGA, R. HINRICHTSEN, AND J. L. VAN HOUTEN. Effect of calmodulin antisense nucleotides on chemoresponse in *Paramecium*. *Chem. Senses* 21: 55–59, 1996.
356. YOUNGENTOB, S. L., AND P. F. KENT. Enhancement of odorant-induced mucosal activity patterns in rats trained on an odorant identification task. *Brain Res.* 670: 82–88, 1995.
357. ZAGOTTA, W. N., AND S. A. SIEGELBAUM. Structure and function of cyclic nucleotide-gated channels. *Annu. Rev. Neurosci.* 19: 235–263, 1996.
358. ZEISKE, E., B. THEISEN, AND H. BREUCKER. The olfactory organ of the hardhead sea catfish, *Arius felis* (L.): gross morphology and fine structure. *Acta Zoologica (Stockholm)* 75: 115–123, 1994.
359. ZENNER, H.-P. Hearing. In: *Comprehensive Human Physiology*, edited by R. Greger and U. Windhorst. Berlin: Springer, 1996, p. 711–727.
360. ZHAINAZAROV, A. B., AND B. W. ACHE. Na<sup>+</sup>-activated nonselective cation channels in primary olfactory neurons. *J. Neurophysiol.* 73: 1774–1781, 1995.
361. ZHAINAZAROV, A. B., AND B. W. ACHE. Odor-induced currents in *Xenopus* olfactory receptor cells measured with perforated-patch recording. *J. Neurophysiol.* 74: 479–483, 1995.
362. ZHAO, H., S. FIRESTEIN, AND C. A. GREER. NADPH-diaphorase localization in the olfactory system. *Neuroreport* 6: 149–152, 1994.
363. ZIEGELBERGER, G., M. J. VAN DEN BERG, K.-E. KAISLING, S. KLUMPP, AND J. E. SCHULZ. Cyclic GMP levels and guanylate cyclase activity in pheromone sensitive antennae of the silkwmoth *Antheraea polyphemus* and *Bombyx mori*. *J. Neurosci.* 10: 1217–1225, 1990.
364. ZIELINSKI, B. S., M. L. GETCHELL, AND T. V. GETCHELL. Ultrastructural evidence for peptidergic innervation of the apical region of frog olfactory epithelium. *Brain Res.* 492: 361–365, 1989.
365. ZIGMAN, J. M., G. T. WESTERMARK, J. LAMENDOLA, E. BOEL, AND D. F. STEINER. Human G<sub>olf</sub>: complementary deoxyribonucleic acid structure and expression in pancreatic islets and other tissues outside the olfactory neuroepithelium and central nervous system. *Endocrinology* 133: 2508–2514, 1993.
366. ZIPPEL, H. P. Historical aspects of research on the vertebrate olfactory system. *Naturwissenschaften* 80: 65–76, 1993.
367. ZIPPEL, H. P., T. LAGO-SCHAAF, AND J. CAPRIO. Ciliated olfactory receptor neurons in goldfish (*Carassius auratus*) partially survive nerve axotomy, rapidly regenerate and respond to amino acids. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* 173: 537–547, 1993.
368. ZIPPEL, H. P., P. W. SORENSEN, AND A. HANSEN. High correlation between microvillous olfactory receptor cell abundance and sensitivity to pheromones in olfactory nerve-sectioned goldfish. *J. Comp. Physiol. A Sens. Neural Behav. Physiol.* 180: 39–52, 1997.
369. ZUFALL, F. Cyclic nucleotide-gated channels, nitric oxide, and neural function. *Neuroscientist* 2: 24–32, 1996.
370. ZUFALL, F., AND S. FIRESTEIN. Divalent cations block the cyclic nucleotide-gated channel of olfactory receptor neurons. *J. Neurophysiol.* 69: 1758–1768, 1993.

371. ZUFALL, F., S. FIRESTEIN, AND G. M. SHEPHERD. Analysis of single cyclic nucleotide-gated channels in olfactory receptor cells. *J. Neurosci.* 11: 3573–3580, 1991.
372. ZUFALL, F., S. FIRESTEIN, AND G. M. SHEPHERD. Cyclic nucleotide-gated ion channels and sensory transduction in olfactory receptor neurons. *Annu. Rev. Biophys. Biomol. Struct.* 23: 577–607, 1994.
373. ZUFALL, F., AND H. HATT. Dual activation of a sex pheromone-dependent ion channel from insect olfactory dendrites by protein kinase C activators and cyclic GMP. *Proc. Natl. Acad. Sci. USA* 88: 8520–8524, 1991.
374. ZUFALL, F., H. HATT, AND S. FIRESTEIN. Rapid application and removal of second messengers to cyclic nucleotide-gated channels from olfactory epithelium. *Proc. Natl. Acad. Sci. USA* 90: 9335–9339, 1993.
375. ZUFALL, F., AND T. LEINDERS-ZUFALL. Identification of a long-lasting form of odor adaptation that depends on the carbon monoxide/cGMP second-messenger system. *J. Neurosci.* 17: 2703–2712, 1997.
376. ZUFALL, F., G. M. SHEPHERD, AND S. FIRESTEIN. Inhibition of the olfactory cyclic nucleotide gated ion channel by intracellular calcium. *Proc. R. Soc. Lond. B Biol. Sci.* 246: 225–230, 1991.
377. ZVIMAN, M., AND H. TI TIEN. Reconstituted olfactory receptors in bilayer lipid membranes. *Bioelectrochem. Bioenerg.* 36: 127–135, 1995.