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A KINETIC MODEL OF THE ODOR RESPONSE IN SINGLE OLFACTORY RECEPTOR NEURONS

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Summary—The detection of odor molecules by olfactory receptors is a biochemical process, but the neural signal is electrical. The transformation of chemical information into a change in membrane potential, i.e. the process of signal transduction, is accomplished in olfactory receptor neurons by a multi-step second messenger pathway resulting finally in the activation of ion channels by cAMP. Many of the biochemical and physiological details of this process are beginning to be appreciated, giving rise to a comprehensive model of the basic mechanisms of olfactory transduction that has much in common with those of other signal transduction systems. One interesting result of these new insights is that the olfactory neuron may act more as a molecule counter than a concentration detector, as had been believed previously.

INTRODUCTION

Although the detection of an odor molecule in the environment is primarily a chemical and biochemical process, the signal which is sent to the nervous system announcing that detection has occurred is electrical: the binding of an odor molecule to a membrane protein receptor must finally result in a change of membrane potential in the sensory neuron. Recently, it has become clear that this is accomplished through a multi-step process involving enzyme cascades, signal amplification, cooperative binding, electrotonic spread of current and the activation of voltage-sensitive ion channels. All of these processes are integrated in the signal transduction function of the olfactory receptor neuron. By recording the ionic currents elicited by odor stimuli it is possible to develop a coherent view of the molecular processes underlying the earliest steps in olfactory perception.

Figure 1 presents a schematic diagram of a vertebrate olfactory receptor neuron. The basic form is of a bipolar neuron with a single dendrite extending from the apical pole of the cell body and an axon projecting from the basal pole. The dendrite is between 10 and 100 mm long and ends in a knob like swelling. Originating from this knob are 6–20 very thin cilia (*ca* 0.2–0.5 μm dia) of variable length. The morphology of this cell is simple and consistent from cell to cell, and even across widely separated species. Functionally it can be thought of as two compartments: the cilia, which are believed to be the main site of odor detection; and the soma/dendrite, containing the voltage-sensitive mechanisms responsible for the generation of the action potential. The remainder of this discussion will center on the ionic currents that directly result from exposure to odor molecules.

THE ODOR RESPONSE IS INITIATED IN THE CILIA

The entire process of odor binding and transduction appears to occur mainly in the specialized cilia which extend from the knob into the mucus [1, 2]. Direct experimental evidence for this is shown in Fig. 2. In this experiment a cell with a particularly long dendrite (about 200 μm) was chosen for recording. This allowed pulses of pressure-ejected odor solution to be directed selectively at either the cilia or soma region of the cell. When the odor stimulus was directed at the cilia a large inward current was elicited [Fig. 2(A)]. The same stimulus failed to induce any current when it was directed at the soma instead [Fig. 2(B)].

If the odor solution was replaced with a high concentration KCl solution (125 mM) the opposite results were obtained: an inward current resulted from pulses directed at the soma but not from those at the cilia [Fig. 2(C)]. Normally KCl would depolarize the cell (i.e.

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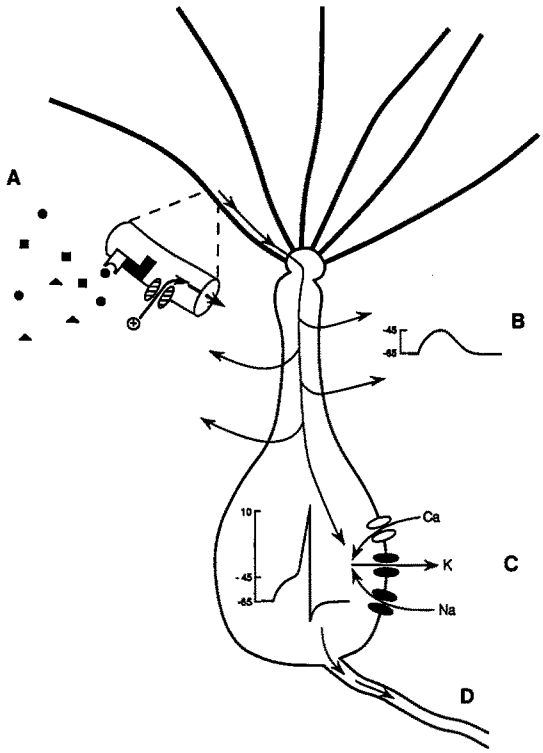

Fig. 1. Schematic of a typical olfactory receptor neuron showing the transduction current pathway. The enzymatic machinery comprising the transduction cascade is located in the cilia (A) and includes a receptor, G-protein, adenylate cyclase and ion channel. This ion channel is the current pathway for the depolarizing current underlying the generator potential (B). This current spreads electrotonically (→) to the soma where it drives the membrane potential to about −45 mV, the threshold for action potential generation. A family of voltage-gated currents in the soma generates the action potential (C) which spreads down the axon (D) to central synapses.

induce an inward current) through leakage channels in the cell membrane. Therefore it appears that leakage channels are present, as expected, in the soma membrane, but they are absent or reduced in the cilia membrane.

This is strong evidence for segregation of function between the two cellular regions. The cilia are clearly involved in odor detection, whereas the role of the soma is to set the resting potential and regulate the electrical activity of the cell. Similar functional differentiation is seen in other sensory cells such as photoreceptors [3, 4] and auditory hair cells [5].

CHARACTERISTICS OF THE ELECTROPHYSIOLOGICAL RESPONSE TO ODOR STIMULATION

The physiological response to odors can best be appreciated by applying very brief pulses (< 100 ms) of odor stimuli directly to an isolated cell. For stimulus pulses this short there is no desensitization and the time course of the stimulus can easily be divorced from that of the response. Experiments utilizing a pressure-ejection system for the rapid application of varying stimulus concentrations are summarized in Fig. 3. A family of responses to increasing concentrations of odor pulses (↓) are shown in Fig. 3(A). The downward deflections of the trace represent inward positive current flow, i.e. depolarizing current. In Fig. 3(B) three responses from another cell to weak, medium and strong stimulation are shown along with the time course of the stimulus solution superimposed (– – –). The time course was determined by including an elevated concentration of K^+ in the odor solution and utilizing the cell's normal response to K^+ as a monitor for the time course and amplitude of the ejected stimulus solution (for details of this method see Refs [2, 6]). Figure 3(C) is a dose–response relation taken from these data, in which the evoked current is plotted as a function of odor concentration.

Several key features of the odor-elicited current response can be seen in these data. First,

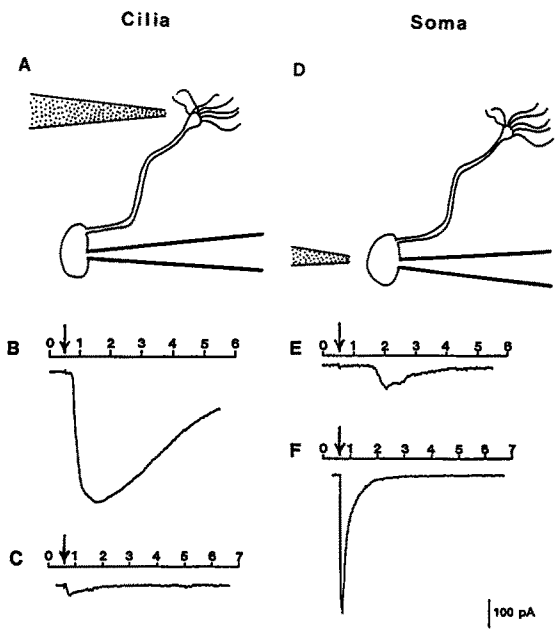

Fig. 2. Segregation of the response to odor and K^+ in different regions of the cell. In cells with longer dendrites it was possible to direct the stimulus to either the cilia or the soma. The diagram is taken from one such experiment. When the odor pulse was directed at the cilia (A) a large inward current was evoked (B). But almost no response was elicited when the stimulus was 100 mM KCl (C). Conversely, an odor pulse directed at the soma (D) elicited no current until some of the stimulus had diffused to the cilia, about 1 s later (E). But a pulse of KCl directed at the soma evoked a large inward current (F).

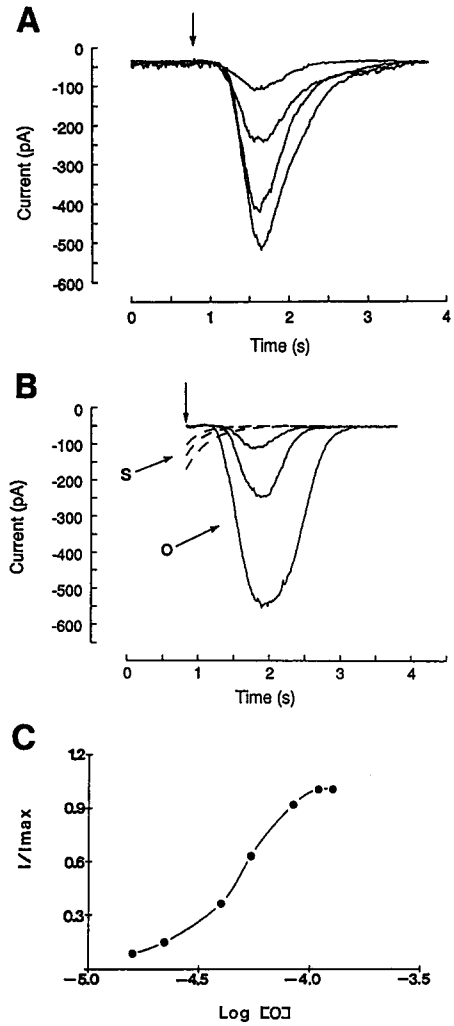

Fig. 3. The physiological response to odors. (A) A family of responses to increasingly strong 50 ms pulses of an odor solution containing 1 mM amyl acetate, acetophenone and cineole, delivered at the arrow. Note the latency of nearly 500 ms, which does not change appreciably over the entire range of responses. (B) Three responses to a weak, a medium and a strong stimulus pulse. The time course of the pressure-ejected stimulus solution for each pulse is shown by (----), marked S. These were determined by including 100 mM KCl in the stimulus solution and monitoring the cells response to the elevated K^+. This portion of the response was then separated from the odor-elicited current (marked O) by computer subtraction methods. Note that the peak of the odor response actually occurs in the virtual absence of any stimulus. (C) Typical dose–response relation for an olfactory receptor neuron. The ordinate is normalized current and the abscissa is the log of the odor concentration. Different cells were responsive over different concentration ranges but the shape of the curve was consistent.

there was a relatively long latency from the arrival of the odor stimulus [↓, Fig. 3(A)] to the initiation of the odor-evoked current. This latency ranged from 150 to 500 ms in different cells and was only slightly sensitive to concentration, decreasing by no more than 20% at saturating stimulus concentrations.

Second, the time course of the odor-elicited current did not reflect the time course of the stimulus [– – –, Fig. 3(B)]. The stimulus attained its peak amplitude rapidly (<20 ms) and decayed away along an approximately exponential time course, with a time constant on the order of 200 ms. By contrast, the odor-elicited current activated along a sigmoidal time course requiring 300–500 ms to reach peak amplitude and decayed exponentially with a time constant of nearly 2 s. Thus, the kinetics of the odor-elicited current were of a different form and were slower by at least an order of magnitude than the time course of the stimulus. One important result of these different kinetics is that the odor response actually occurred in the virtual absence of the stimulus, which had diffused away completely by the peak of the odor-elicited current. This is shown clearly in Fig. 3(B) where the time course of the stimulus and the odor current are compared for three stimulus levels.

Third, from the dose–response data it appears that the dynamic operating range of olfactory receptors is rather narrow. Typically saturation of the response was achieved within only a log unit of concentration change. In different receptor cells the range of absolute concentration sensitivity may vary (i.e. the curve may shift left or right along the concentration axis) but the steepness (i.e. the shape of the relation) is consistent. This might be compared to photoreceptors which are sensitive to light intensities over 3–4 log units [7].

These response characteristics led us to consider exactly what primary feature, or features, of the stimulus are being extracted by the olfactory neuron. Specifically, since the response is so much slower than the stimulus, it seems that the cell may be integrating its response over some time interval, acting in this respect as a molecule counter rather than a concentration detector. That is, the olfactory receptor neuron may respond not to the concentration of odor, but to the number of odor molecules detected over some time period, i.e. the "flux" of molecules. From a physiological point of view this is a more meaningful measure since it is not possible for a receptor fixed in the surface membrane of a cell to sample a volume extending some arbitrary distance from that surface. The receptor can only capture single odor molecules which approach sufficiently close. It is true, of course, that in the steady-state flux will be a simple function of concentration, but during the dynamic response time of

the cell these two measures may not be equivalent; in which case the cell is forced to make judgments about changes in concentration based on numbers of molecules captured (i.e. bound) over some sampling period.

To test the possibility that olfactory neurons respond to molecular flux we performed experiments similar to that depicted in Fig. 4. Pulses of odor of varying durations but equal peak concentrations were delivered to a receptor neuron. The initial downward deflections of the current traces, marked with an S, show the time course and amplitude of the stimulus as determined by the cell's response to the K^+ ions in the solution. Note that the pulses all attained the same peak amplitude, which is a measure of the concentration, but each was of a different duration. One might think of the integral under these curves as a measure of the number of odor molecules delivered to the cell during a particular pulse. The second deflections in the current traces, marked with an R, are the odor-elicited currents. Clearly the magnitude of the response followed the integral of the stimulus curves, i.e. the number of molecules, rather than their amplitude (the concentration), suggesting that the receptor neuron measures not only concentration but integrates concentration information over some time period. From other experiments we have determined this integration time to be approx. 750–1000 ms. In a rigorous mathematical model of an analogous problem in bacterial chemotaxis Berg and Purcell [8] derived a period of 0.7–1 s as optimal for a cell to estimate concentration changes from independent samples of molecular flux.

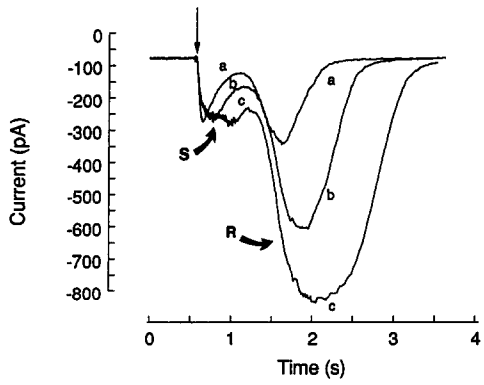

Fig. 4. The olfactory receptor neuron integrated over time. Three responses are shown. The stimulus, represented by the K^+ currents (the first downward deflection in the traces) was delivered in pulses of increasing duration, while the pressure was adjusted to maintain the same peak concentration. The odor-elicited currents followed the integral of the stimulus and not the maximal concentration. Pulse durations were: (a) 50 ms; (b) 200 ms; (c) 500 ms.

A MODEL OF OLFACTORY TRANSDUCTION FROM THE KINETICS OF THE ODOR RESPONSE

Based on these considerations, we conclude that a comprehensive model of olfactory transduction must account for these several features of the cell's physiological response. We were attracted to a mechanism which made use of a multi-step second messenger system including an integrating step and an amplification step. Considerable biochemical evidence for a G-protein based cAMP second messenger system has been amassed over the past few years (see Ref. [9] for a recent review) and we have endeavored to provide correlative physiological evidence in individual cells [10]. Our strategy has been first to characterize the components of this intracellular enzyme cascade, and second to identify the particular steps which shape the response to odors.

Figure 5 shows the proposed scheme graphically (A) and as a series of equations based on formalisms developed for enzyme kinetics (B). The evidence for the various components of the cascade include the following:

1. A family of genes coding for the receptor protein has been identified [11] and this receptor appears to be closely related to other G-coupled receptors with 7 transmembrane domains [12].
2. The nonhydrolyzable analogue of GTP-g-S prolongs the odor response, and GDP-b-S blocks it, demonstrating a critical role for G-proteins in the transduction pathway. An olfactory specific G-protein has been identified [13].
3. Increasing intracellular cAMP induces a current with the same properties as the odor-sensitive current, and saturating concentrations of cAMP occlude the normal odor response. Production of cAMP in an odor-dependent manner has been demonstrated [14–16] and an olfactory specific adenylyl cyclase has been identified [17].
4. Phosphodiesterase inhibitors, such as IBMX, prolong the duration of the response to very brief pulses of odor [10]. This shows that the termination of the odor response is due primarily to the hydrolysis of cAMP.
5. An odor-sensitive ion channel is also gated directly by intracellular cyclic nucleotides [18, 19]. This ion channel is responsible

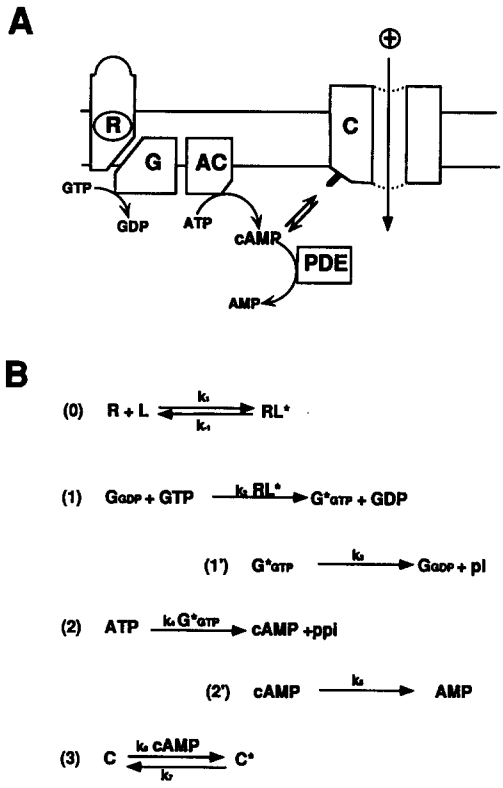

Fig. 5. Schematic (A) of the main components of the second messenger system mediating olfactory transduction (TOP). This scheme is also shown as a series of equations (B) depicting each step in the cascade (see the text).

for the depolarizing current which initiates the cell's electrical response.

In this model there are three main steps leading to the activation of the odor-induced current (1, 2 and 3) and two steps (1′, 2′) which determine its decay. The onset kinetics are determined by the rate constants k_2, k_4 and k_6 (k_1, which characterizes the interaction between the receptor and odor ligand is assumed to be very fast). The decay kinetics are governed by k_3, k_5 and k_7. We have attempted to determine which of these steps is responsible for the physiological features of the odor response by developing a computational model based on these equations and constrained by experimental data such as that noted above.

From this analysis we have concluded the following: (1) the major part of the response latency appears to be the loading of the G-protein, i.e. step 1 (rate constant k_2); (2) the narrow dynamic range is due to high gain amplification at step 2 (rate constant k_4), which is the production of cAMP by the G-protein activated adenylate cyclase; and (3) the sigmoidal time course of the current activation is due to the requirement for cooperative activity of at least three molecules of cAMP to open the channel at step 3 (rate constant k_6) [18–20].

In this model the accumulation of activated G-protein serves as the integrating step, i.e. the molecular counter. But once the olfactory cyclase, perhaps the most active cyclase in the nervous system [17], is activated there is a rapid and significant amplification of the signal. This accounts for the narrow operating range seen in the dose–response relations.

Several important questions regarding olfactory transduction are raised by this model. If olfactory receptor neurons are sensitive to molecular flux and "count" molecules, then what is their lower limit of sensitivity? At least theoretically the detection of single odor molecules is possible. By analogy photoreceptors are known to have the capability of detecting single photons of light [21]. Experiments to determine olfactory receptor sensitivity are underway in our laboratory.

It should also be noted that a scheme such as that proposed here offers several possible mechanisms for adaptation and other feedback regulation. The data in this area remain sketchy, but clearly modulation of any of these key rate constants, e.g. by phosphorylation, would significantly alter the odor response.

Finally, it is worth considering the appropriate dimensions of the stimulus. Commonly olfactory stimuli are reported in molar units. For exposures which last more than a few seconds this is sufficiently accurate, although the response of cells to these long stimulations is probably complicated by adaptation effects. For briefer stimuli the more accurate measure would be "flux" in units such as mol l^{-1} s^{-1}. The surface area of the receptive membrane is also a critical factor, so that the best units might be molecules cm^{-3} s^{-1}. In any case, the temporal dimension is critical to understanding the mechanisms underlying olfactory transduction and should be carefully considered in discussions of information processing in this system.

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