Adenylate cyclase mediates olfactory transduction for a wide variety of odorants

(olfaction/cyclic AMP/cilia/forskolin/sensory transduction)

GRAEME LOWE, TADASHI NAKAMURA*, AND GEOFFREY H. GOLD

Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104

ABSTRACT   An odor-stimulated adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] is thought to mediate olfactory transduction in vertebrates. However, it is not known whether the adenylate cyclase serves this function for all odorants or for only certain classes of odorants. To investigate this question, we have compared the abilities of 35 odorants to stimulate the adenylate cyclase and to elicit an electrophysiological response. We report a strong positive correlation between the magnitude of adenylate cyclase stimulation and the summated electrical response of the olfactory epithelium (electro-olfactogram) evoked by individual odorants. We also show that the adenylate cyclase stimulator forskolin equally attenuates the electro-olfactogram response for all odorants tested. These data provide evidence that the adenylate cyclase mediates transduction for a wide variety of odorants.

Olfactory receptor cells respond to odors by an increase in membrane conductance, leading to membrane depolarization and the generation of action potentials (reviewed in refs. 1 and 2). The mechanism(s) by which odors regulate the membrane conductance is not known with certainty; however, a considerable body of evidence indicates that cyclic AMP serves as an intracellular messenger in this process. For example, olfactory cilia contain an odor-stimulated adenylate cyclase (3–5). In addition, we have described a cyclic nucleotide-gated conductance that could translate the odor-induced rise in cyclic AMP concentration into a conductance increase (6). The involvement of the cyclic AMP pathway is further suggested by the effects of pharmacological agents on the electrical responses to odors, measured with transepithelial voltage and current recordings (7–9).

A key question concerning the cyclic AMP mechanism is whether it mediates transduction for all odorants or only certain classes of odorants. We have addressed this question by comparing the abilities of different odorants, at a fixed concentration, to stimulate adenylate cyclase and to elicit an electrophysiological response. For the adenylate cyclase measurements we used data published by Sklar *et al.* (4), who used cilia purified from the bullfrog olfactory epithelium. For the electrophysiological measurements, we recorded, also in the bullfrog, the transepithelial voltage response to odorants [the electro-olfactogram, or EOG (10)]. The EOG is an extracellular field potential produced by the odor-induced generator currents in the olfactory receptor neurons (1, 11, 12). Because both the adenylate cyclase and the EOG measurements summate the activity of receptor neurons throughout the olfactory epithelium, we would expect a positive correlation between the magnitudes of adenylate cyclase stimulation and the EOG for all odorants that are detected via the adenylate cyclase pathway. We report a

strong positive correlation between the magnitudes of adenylate cyclase stimulation and the EOG responses to 35 odorants.

We have further investigated the generality of the adenylate cyclase pathway by observing the effects of forskolin on the EOG responses to a variety of different odorants. Forskolin stimulates the adenylate cyclase by a direct effect on the enzyme (reviewed in refs. 13 and 14) and therefore, according to the cyclic AMP model (6), should decrease odor responses both by its effect on the adenylate cyclase (by reducing the number of enzyme molecules that may then be activated by odors) and by its effect on the basal cyclic AMP concentration (by increasing basal cyclic AMP concentration, forskolin will partially saturate the ciliary conductance, thus decreasing odor-induced increments in membrane conductance). We report that forskolin equally attenuates the responses to all odorants tested. Thus, both sets of data presented here are consistent with the hypothesis that the cyclic AMP pathway mediates transduction for a wide variety of odorants.

MATERIALS AND METHODS

Bullfrogs (*Rana catesbeiana*) were obtained from Connecticut Valley Biological (Southampton, MA), Western Scientific (Sacramento, CA), and W. A. Lemberger (Oshkosh, WI). Odorants used were of the highest purity commercially available; some were generously provided by International Flavors and Fragrances (Union Beach, NJ). Forskolin and 1,9-dideoxyforskolin were purchased from Calbiochem.

The EOG was measured from excised bullfrog olfactory epithelia, which were mounted in a perfusion chamber. Frogs were decapitated and pithed, and the dorsal surface of the nasal cavity was soaked in Ringer's solution for *ca.* 2 hr at room temperature. The 2-hr soak in Ringer's solution was found to give EOG responses of larger amplitudes. The olfactory epithelium was then dissected away from the remaining portion of the skull and mounted, apical (ciliated) surface up, in a Plexiglas recording chamber (15), and the edges were sealed with vacuum grease. The apical surface was continuously superfused with Ringer's solution via a plug, which sealed the chamber from above. The plug had a central port for delivery of fresh Ringer's solution (flow rate 2.0 ml/min) and a concentric array of peripheral exit ports to maintain an approximately radial flow pattern. The exposed area of the epithelium was 12 mm^2, which utilized most of the dissected tissue. The Ringer's solution contained 100 mM NaCl, 2.5 mM KCl, 10 mM NaHCO$_3$, 1 mM CaCl$_2$, 1 mM MgSO$_4$, and 10 mM glucose, in equilibrium with 98.5% O$_2$/1.5% CO$_2$, at pH 7.6.

Abbreviation: EOG, electro-olfactogram.
*Present address: Department of Applied Physics and Chemistry, University of Electro-Communications, 1-5-1 Chofugaoka, Chofu-shi, Tokyo, Japan.

Odorant stimulation was performed by switching a calibrated volume of the odorant, dissolved in Ringer's solution, into the superfusion line. All odorants were applied at a concentration of 100 μM to mimic as closely as possible the conditions used in the adenylate cyclase assay (4). The use of aqueous stimulation and superfusion offers several advantages over vapor-phase stimulation: (i) pharmacological agents, such as forskolin, may be introduced without altering the thickness of the aqueous layer overlying the epithelium; (ii) the odorant concentration in the aqueous layer is obtained directly, without reference to air/water partition coefficient data; and (iii) the increased thickness of the aqueous layer will improve spatial summation of the EOG by reducing the tangential resistance of the aqueous layer. [Describing the epithelium as a resistive sheet with transverse resistance of 100 ohm·cm² (9), in contact with the mucus layer, a sheet of resistivity 100 ohm·cm and 30 μm in thickness, we estimate the length constant for passive spread of field potentials to be 500 μm, which will be increased about 5-fold by the aqueous layer in our recording chamber.] The stimulus volume was 80 μl and the flow rate was 2.0 ml/min, so the stimulus duration was ≈2.4 s. Odorant solutions were prepared at 100 μM by two 10-fold dilutions into Ringer's solution of a stock 10 mM odorant/water mixture. Stimuli were injected into an 80-μl loop of Teflon tubing and switched into the flow by a six-port sampling valve. Ag/AgCl electrodes were placed in contact with the bottom of the chamber (reference) and with the superfusing solution downstream from the chamber (recording). The output was amplified and low-pass-filtered (8-pole Bessel; corner frequency = 40 Hz).

An additional three-port valve was placed upstream of the sampling valve to allow the superfusing solution to be switched to one containing forskolin. Ringer's solution with forskolin was made by dilution of a 20 mM forskolin stock in ethanol, giving a final ethanol concentration of 0.86 mM. Switching to 0.86 mM ethanol alone gave a small transient negative shift in transepithelial potential (magnitude ca. 25% of the maintained forskolin-induced shift), followed by slow recovery to baseline, and only a slight attenuation (6%) of subsequent odorant responses with no change in the shape of the response. To minimize exposure to forskolin, stimuli were applied within 10 s after the attainment of the new baseline voltage or, in the case of drift, after the attainment of a constant slope. Washout with Ringer's solution was initiated ca. 2 min after stimulation, and a slow exponential decay (time constant of 1.6 min at 22°C) back to the original baseline followed. We used only preparations in which responses to the odorant 2-hexylpyridine at 100 μM, either measured directly or estimated from the responses to other odorants (see below), exceeded 2.5 mV. Smaller amplitudes were correlated with a larger amount of scatter in the data.

The observed EOG responses were ≈8 s in duration at one-half peak amplitude. The interstimulus interval was 5–10 min and was long enough to ensure that each response was not diminished by desensitization caused by the preceding stimulus. All EOG responses were negative in polarity (apical with respect to basal surface of the epithelium), with amplitudes as large as 6 mV [mean = 2.8 mV for 21 responses to 2-hexylpyridine (100 μM), the most potent odorant]. Positive EOG responses, which indicate contributions from electrogenic activity of the supporting cells (16), were not observed.

RESULTS

We recorded the transepithelial voltage responses (EOGs) to 35 different odorants. All odorants were applied dissolved in Ringer's solution at a concentration of 100 μM, to mimic as closely as possible the conditions used in the adenylate cyclase measurements reported by Sklar et al. (4). Fig. 1 shows consecutive EOG responses to five different odorants.
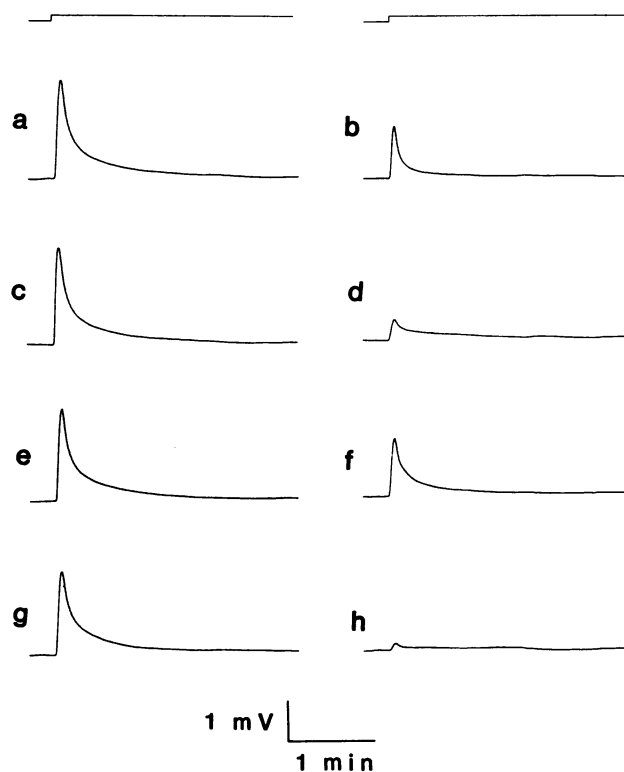

FIG. 1. Successive EOGs elicited by transient exposure of a frog olfactory epithelium to the odorants 2-hexylpyridine (traces a, c, e, and g), isoamyl acetate (trace b), amyl salicylate (trace d), geraniol (trace f), and triethylamine (trace h). Similar waveforms were elicited by other odorants. The responses were negative in polarity (apical with respect to basal sides of the epithelium) but are displayed negative-upwards, as is commonly done. The chronological order of the traces was a, b, c, d, e, f, g, with 5–6 min between each stimulus. The top traces show the position of the sampling valve used to inject a pulse of odorant into the flowline. Therefore, the upward step in those traces indicates the approximate time of initiating the odor stimulus. The stimulus duration is not shown but can be estimated from the perfusion flow rate and the stimulus volume (see Materials and Methods).

The stimuli alternated between a floral odorant, 2-hexylpyridine (traces a, c, e, g), and four other odorants: isoamyl acetate (fruity, trace b), amyl salicylate (floral, trace d), geraniol (floral, trace f), and triethylamine (putrid, trace h). Although all odorants were applied at 100 μM, the response amplitudes for the four odorants in the right half of the figure varied between 0.5 and 1.5 mV. In contrast, the amplitude of the response to 2-hexylpyridine (left half of figure) remained nearly constant at about 2.5 mV. Because the absolute magnitude of the response to a single odorant varied between different preparations, the amplitude of the response to each odorant is expressed as a fraction of the amplitude of the response to 2-hexylpyridine in each preparation. This normalization procedure was also used to compensate for the gradual loss of sensitivity in a preparation over time; the response to a single odorant typically decreased about 20% per hour. The validity of this normalization procedure was demonstrated by the constancy of the normalized responses to 18 different odorants; the normalized response varied <10% over 1 hr in individual preparations (tested for 7 odorants), and its standard deviation for each odorant was <35% of the mean when different preparations (up to 8) were included (tested for 13 odorants).

To determine whether the magnitudes of these electrophysiological responses were correlated with adenylate cyclase activity, the normalized EOG amplitudes for each of 35 odorants were compared with the magnitudes of their ade-

Neurobiology: Lowe *et al.*
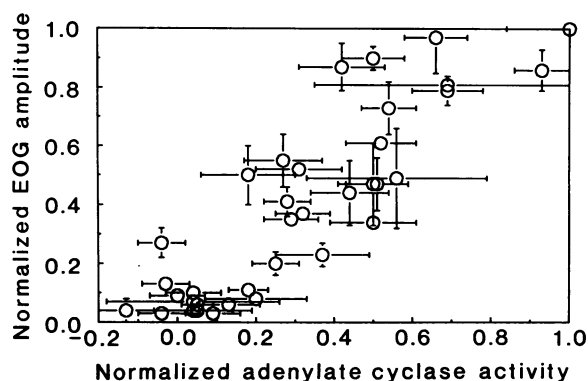
Proc. Natl. Acad. Sci. USA 86 (1989)   5643

FIG. 2. Relationship between normalized EOG amplitude and adenylate cyclase activity (cyclase data taken from ref. 4). EOGs were recorded as described in Fig. 1, with odorant stimulation alternating between the test odorant and the normalizing reference odorant, 2-hexylpyridine. The normalized EOG response for each odorant was computed by dividing the test odorant response amplitude by the amplitude of the 2-hexylpyridine response, the latter obtained by linearly interpolating between the 2-hexylpyridine responses immediately before and after the test odorant. The results are listed in Table 1 and are plotted in this figure against the relative cyclase stimulation by each odorant (published in ref. 4), normalized to the stimulation produced by 2-hexylpyridine.

nylate cyclase stimulation (adenylate cyclase measurements taken from ref. 4). The data are plotted in Fig. 2 and indicate a strong positive correlation between EOG amplitude and adenylate cyclase stimulation (correlation coefficient = 0.87; the numerical values are listed in Table 1). The 35 odorants chosen represented the full range of adenylate cyclase stimulation observed in ref. 4, as well as all of the odor qualities tested—i.e., fruity, floral, minty, herbaceous, putrid, and solvent.

To further test the role of adenylate cyclase in olfactory transduction, we investigated the effect of forskolin on the EOG responses to 16 odorants. As explained above, forskolin should decrease the amplitudes of all odor responses that are mediated via the adenylate cyclase pathway. Fig. 3 *Upper* shows the protocol for this experiment. First, an odor response was measured in control Ringer's solution (arrow, trace a), and then the superfusate was switched to Ringer's solution containing 1 μM forskolin. Forskolin induced a stable shift in potential, which presumably reflected a maintained increase in cyclic AMP concentration. For trace b, this protocol was repeated, and a second odor stimulus was given after a steady forskolin effect was obtained (second arrow). The odor response in the presence of forskolin, measured relative to the elevated baseline, was reduced in amplitude and slower in time-course. As expected, the degree of attenuation increased monotonically with forskolin concentration, with complete abolition of the odor response occurring by about 10 μM (data not shown). The effect of forskolin appears to reflect specific activation of adenylate cyclase, because the inactive derivative 1,9-dideoxyforskolin had no effect either on the EOG baseline or on the response amplitude or time course at 1 μM (data not shown).

The attenuation of the peak response amplitude induced by 1 μM forskolin was measured for 16 odorants and was plotted as a function of the normalized EOG amplitude for each odorant (Fig. 3 *Lower*). The odorants chosen represent the full range of normalized EOG amplitudes and, therefore, the magnitude of adenylate cyclase stimulation. The forskolin concentration of 1 μM was chosen because it produced moderate attenuation that could be reliably measured, even for odorants with small control amplitudes. There are no significant deviations by individual odorants from the mean attenuation (0.33), and there is no significant correlation

Table 1. Normalized EOG amplitudes and adenylate cyclase stimulation for odorants at 100 μM

Odorant	Normalized EOG		Normalized cyclase stimulation	
Acetic acid	0.04 ± 0.04	(2)	−0.13 ± 0.32	(3)
Acetophenone	0.41 ± 0.05	(5)	0.28 ± 0.06	(3)
Amyl salicylate	0.23 ± 0.04	(5)	0.37 ± 0.12	(2)
Benzaldehyde	0.20 ± 0.04	(9)	0.25 ± 0.06	(4)
1-Butanol	0.04 ± 0.03	(3)	0.04 ± 0.10	(3)
D-Carvone	0.79 ± 0.05	(6)	0.69 ± 0.09	(7)
L-Carvone	0.81 ± 0.03	(4)	0.69 ± 0.34	(6)
Chloroform	0.04 ± 0.03	(4)	0.05 ± 0.02	(3)
Cineole (eucalyptol)	0.87 ± 0.08	(17)	0.42 ± 0.11	(4)
Cinnamaldehyde	0.37 ± 0.01	(4)	0.32 ± 0.07	(4)
Citralva	0.86 ± 0.07	(3)	0.93 ± 0.07	
Citronellal	0.61 ± 0.02	(4)	0.52 ± 0.09	(3)
Coniferan	0.49 ± 0.17	(5)	0.56 ± 0.23	(2)
Decanal	0.34 ± 0.01	(4)	0.50 ± 0.11	(2)
3,7-Dimethyl-1-octanol	0.52 ± 0.02	(4)	0.31 ± 0.11	(3)
Ethanol	0.03 ± 0.00	(2)	0.09 ± 0.07	(3)
Ethyl vanillin	0.13 ± 0.03	(3)	−0.03 ± 0.06	(5)
Eugenol	0.44 ± 0.11	(16)	0.44 ± 0.10	(5)
Furfuryl mercaptan	0.55 ± 0.10	(5)	0.27 ± 0.10	(3)
Geraniol	0.73 ± 0.09	(12)	0.54 ± 0.07	(3)
Helional	0.47 ± 0.14	(7)	0.50 ± 0.09	(5)
2-Hexylpyridine	1.00		1.00 ± 0.15	(3)
β-Ionone	0.47 ± 0.09	(24)	0.51 ± 0.08	(5)
Isoamyl acetate	0.50 ± 0.10	(13)	0.18 ± 0.12	(8)
2-Isobutyl-3-methoxypyrazine	0.90 ± 0.04	(2)	0.50 ± 0.08	(10)
Isoeugenol	0.35 ± 0.03	(5)	0.29 ± 0.07	(3)
Limonene	0.06 ± 0.02	(3)	0.05 ± 0.04	(5)
Lyral	0.27 ± 0.05	(4)	−0.04 ± 0.06	(2)
Menthone	0.97 ± 0.12	(22)	0.66 ± 0.08	(4)
Phenethyl alcohol	0.11 ± 0.03	(4)	0.18 ± 0.05	(3)
Phenethylamine	0.09 ± 0.03	(3)	0.00 ± 0.07	(3)
α-Pinene	0.08 ± 0.02	(12)	0.20 ± 0.13	(3)
Pyridine	0.07 ± 0.01	(2)	0.04 ± 0.22	(4)
Pyrrolidine	0.03 ± 0.00	(2)	−0.04 ± 0.06	(2)
Toluene	0.06 ± 0.02	(3)	0.13 ± 0.08	(3)
Triethylamine	0.10 ± 0.02	(4)	0.04 ± 0.07	(5)

Entries are mean ± SD (number of measurements). The adenylate cyclase measurements were taken from ref. 4 and were normalized to the stimulation elicited by 2-hexylpyridine.

between the normalized EOG response and the magnitude of forskolin-induced attenuation for each odorant; i.e., forskolin equally attenuates the responses to all odorants tested. The same result was obtained when we measured the effect of 1 μM forskolin on the initial rate of rise of the odor response. The initial rate of rise is perhaps a more direct measurement of the output of the transduction process, since the peak response amplitude may be a function of both the rising and falling phases of the odor response.

Two odorants, isobutyric acid and isovaleric acid, could not be included in evaluating the correlation between the EOG and adenylate cyclase because the normalized EOG amplitudes for these odorants were highly variable, occasionally increasing from less than 0.1 to as much as 0.5 during a single experiment. The largest normalized EOG values would constitute a significant deviation from the EOG–adenylate cyclase correlation, because of the small magnitude of their adenylate cyclase stimulation (4). Isobutyric acid and isovaleric acid also differed from the other odorants in terms of their forskolin effects; although both odorants exhibited the same attenuation of initial response slope and peak response amplitude as did the other odorants (Table 2), their response duration was not prolonged by forskolin, as
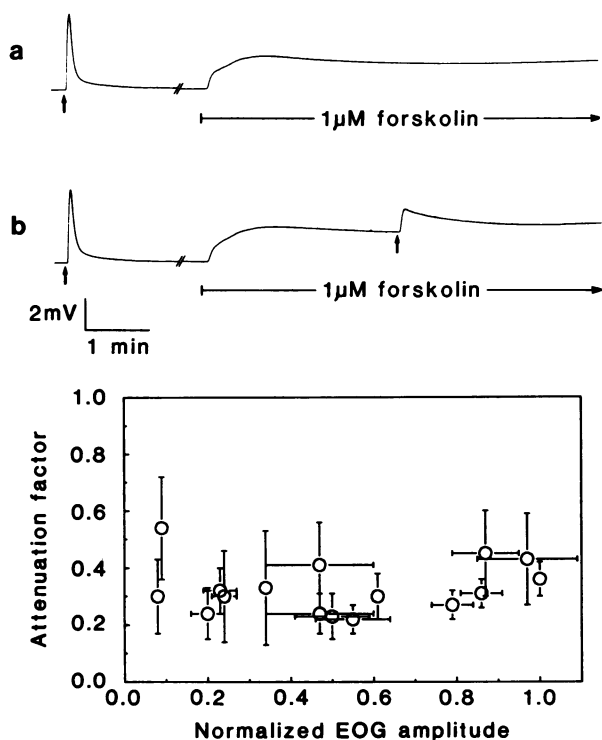
FIG. 3. The effect of forskolin on the EOG. (*Upper*) Trace a shows a control response to 100 μM 2-hexylpyridine in Ringer's solution, followed by a change to prolonged superfusion with 1 μM forskolin, causing a maintained shift in baseline voltage. Trace b shows another control response to 100 μM 2-hexylpyridine recorded in normal Ringer's solution, followed by an attenuated response to 2-hexylpyridine obtained during superfusion with 1 μM forskolin. The effect of forskolin was reversible for exposures of up to 12 min. (*Lower*) Relationship between forskolin-induced attenuation (the ratio of the EOG during and before forskolin application) and the normalized EOG responses for 16 odorants (values listed in Table 2).

was observed for the other odorants (for example, in Fig. 3 the duration at half-maximal amplitude of the response to 2-hexylpyridine was increased from 5.8 s to 55 s). Thus, it

Table 2. Attenuation of EOG responses by forskolin

Odorant	Amplitude ratio
Amyl salicylate	0.32 ± 0.08 (6)
Benzaldehyde	0.24 ± 0.09 (5)
D-Carvone	0.27 ± 0.05 (5)
Cineole	0.45 ± 0.15 (9)
Citralva	0.31 ± 0.05 (9)
Citronellal	0.30 ± 0.08 (6)
Decanal	0.33 ± 0.20 (7)
Furfuryl mercaptan	0.22 ± 0.05 (5)
Helional	0.24 ± 0.07 (5)
2-Hexylpyridine	0.36 ± 0.06 (7)
β-Ionone	0.41 ± 0.15 (6)
Isoamyl acetate	0.23 ± 0.08 (5)
Isobutyric acid	0.23 ± 0.09 (7)
Isovaleric acid	0.14 ± 0.11 (2)
Lyral	0.30 ± 0.16 (5)
Menthone	0.43 ± 0.16 (6)
Phenethylamine	0.54 ± 0.18 (5)
α-Pinene	0.30 ± 0.13 (7)

Data are expressed as the mean ± SD (number of measurements) of the ratio of the amplitude of the response to each odorant recorded during superfusion with 1 μM forskolin to that immediately preceding superfusion with the drug and are plotted against the normalized EOG amplitude in Fig. 3 *Lower* (except for isobutyric acid and isovaleric acid, for which accurate normalized EOG amplitudes could not be determined; see text).

appears that in some respects, isobutyric acid and isovaleric acid differ from the other odorants tested, although our data do not rule out the possibility that these two odorants are transduced via the adenylate cyclase pathway.

DISCUSSION

A unique problem posed by the olfactory system is to understand how it detects and distinguishes between the enormous variety of odorous compounds. It has often been suggested that multiple transduction mechanisms are needed to accomplish this. Consequently, a key issue concerning the cyclic AMP mechanism is whether it mediates transduction for all odorants or only for certain classes of odorants. We have investigated this question by studying the EOG because, by virtue of being a summated response, the EOG provides information about all classes of receptor cells. Consequently, the EOG provides the most suitable electrophysiological measurement for investigating the generality of olfactory transduction mechanisms. Also, by virtue of being a summated response, the EOG is analogous to biochemical measurements, such as the adenylate cyclase assay, allowing a direct comparison between electrophysiological and biochemical data.

The generality of the adenylate cyclase mechanism was first investigated by Sklar et al. (4), who measured the abilities of 65 different odorants to stimulate the adenylate cyclase. They found that the magnitude of adenylate cyclase stimulation elicited by individual odorants, all applied at the same concentration, varied from a maximum of 1.6 times basal activity to a level indistinguishable from basal activity.[†] Because many of the odorants tested failed to elicit significant stimulation, they suggested that the adenylate cyclase pathway does not mediate transduction for all odorants (4). However, the variations in adenylate cyclase activity actually support the involvement of this enzyme in mediating transduction for a wide variety of odorants, because of the direct correlation between cyclase activity and the EOG. Consequently, these variations may reflect differences in the numbers of receptor cells that respond to each odorant, rather than the existence of other transduction mechanisms. This interpretation predicts that, in the bullfrog, receptor cells that respond to many putrid odorants (e.g., pyrrolidine) and organic solvents (e.g., toluene) are 10- to 20-fold less abundant than receptors that respond to many floral and fruity odorants (e.g., geraniol and citralva). This interpretation also predicts that, with improved techniques, adenylate cyclase stimulation should be detectable for the odorants that failed to evoke significant stimulation. These conclusions may also apply to mammals, because the rat adenylate cyclase exhibits an odorant sensitivity profile similar to that of the bullfrog (21).

The equal attenuation of all EOG responses by forskolin is also consistent with the adenylate cyclase mediating transduction for all odorants tested. The complete absence of an odor response in the presence of high concentrations of forskolin might itself be interpreted as evidence against the existence of other transduction mechanisms. However, this is not necessarily the case, because the high ciliary conductance induced by high concentrations of forskolin should mask, by passive shunting, all other odor-regulated currents. Nevertheless, the forskolin data do indicate that all cells which respond to the odorants tested contain both a forskolin-sensitive adenylate cyclase and a cyclic nucleotide-

[†]The 1.6-fold increase in adenylate cyclase activity observed in ciliary preparations must underestimate the magnitude of the increase in individual cells, since the basal ciliary activity is contributed by all cells, whereas the odor-stimulated activity must come from only a subset of receptor cells (17).

Neurobiology: Lowe *et al.*

Proc. Natl. Acad. Sci. USA 86 (1989) 5645

regulated conductance. The correlation between adenylate cyclase stimulation and the EOG indicates that if other mechanisms, such as odor-stimulated phospholipase C activity (18), contribute to olfactory transduction, they must make a similar contribution to the responses to all odorants, rather than augmenting or replacing the adenylate cyclase pathway for only certain classes of odorants.

The attenuation of the EOG by forskolin that we have described may seem to contradict a recent report (9) that forskolin enhances EOG responses measured under transepithelial voltage clamp. However, in that study, enhancement of the EOG by forskolin was observed only after forskolin had been washed out and the EOG baseline had returned to its pretreatment level. This after-effect of forskolin is more difficult to interpret than the immediate effect we have studied, so we believe our data provide a more critical test of the role of adenylate cyclase in olfactory transduction. Another limitation of earlier pharmacological studies is that, for most of the agents used, it was not possible to unambiguously predict the effects of the agents on EOG amplitude. For example, phosphodiesterase inhibitors, used in refs. 7 and 8, could either increase or decrease EOG amplitude, depending on whether their dominant effect is to increase the basal cyclic AMP concentration or to enhance the odor-induced increment in cyclic AMP concentration. Thus, although the observation that phosphodiesterase inhibitors reduce EOG amplitude (7, 8) suggests the involvement of cyclic AMP in olfactory transduction, it fails to specify the nature of that involvement. In contrast, our study focused on an agent that makes an unambiguous and testable prediction, and included a variety of odorants, thus demonstrating the generality of the effects observed.

The overall conclusion of our study is that the adenylate cyclase pathway mediates, or makes an equal contribution to mediating, olfactory transduction for a wide variety of odorants. This is in contrast to taste receptor cells, which appear to utilize different transduction mechanisms for different taste qualities (19, 20). Consequently, our data support the view that olfactory receptor cells derive their odorant specificities from a family of receptor proteins (1), all of which initiate an electrical response via activation of the adenylate cyclase and subsequent opening of cyclic nucleotide-gated ion channels.

1. Lancet, D. (1986) *Annu. Rev. Neurosci.* **9,** 329–355.
2. Getchell, T. V. (1986) *Physiol. Rev.* **66,** 772–817.
3. Pace, U., Hanski, E., Salomon, Y. & Lancet, D. (1985) *Nature (London)* **316,** 255–258.
4. Sklar, P. B., Anholt, R. R. H. & Snyder, S. H. (1986) *J. Biol. Chem.* **261,** 15538–15543.
5. Shirley, S. G., Robinson, C. J., Dickinson, K., Aujla, R. & Dodd, G. H. (1986) *Biochem. J.* **240,** 605–607.
6. Nakamura, T. & Gold, G. H. (1987) *Nature (London)* **325,** 442–444.
7. Minor, A. V. & Sakina, N. L. (1973) *Neirofiziologiya* **5,** 415–422.
8. Menevse, A., Dodd, G. & Poynder, T. M. (1977) *Biochem. Biophys. Res. Commun.* **77,** 671–677.
9. Persaud, K. C., Heck, G. L., DeSimone, S. K., Getchell, T. V. & DeSimone, J. A. (1988) *Biochim. Biophys. Acta* **944,** 49–62.
10. Ottoson, D. (1956) *Acta Physiol. Scand.* **43,** 167–181.
11. Ottoson, D. (1971) *Handbook of Sensory Physiology: Chemical Senses*, ed. Beidler, L. M. (Springer, Berlin), Vol. 4, pp. 95–131.
12. Getchell, T. V. (1974) *J. Neurophysiol.* **37,** 1115–1130.
13. Seamon, K. B. & Daly, J. W. (1981) *J. Cyclic Nucleotide Res.* **7,** 201–224.
14. Seamon, K. B. & Daly, J. W. (1986) *Adv. Cyclic Nucleotide Protein Phosphorylation Res.* **20,** 1–150.
15. Gold, G. H. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1150–1154.
16. Takagi, S. F., Aoki, K., Iino, M. & Yajima, T. (1969) in *Olfaction and Taste*, ed. Pfaffmann, C. (Rockefeller Univ. Press, New York), pp. 92–108.
17. Duchamp, A., Revial, M. F., Holley, A. & MacLeod, P. (1974) *Chem. Senses Flavour* **1,** 213–233.
18. Huque, T. & Bruch, R. C. (1986) *Biochem. Biophys. Res. Commun.* **137,** 36–42.
19. Teeter, J. & Gold, G. H. (1988) *Nature (London)* **331,** 298–299.
20. Kinnamon, S. C. (1988) *Trends Neurosci.* **11,** 491–496.
21. Sklar, P. B., Pearson, R. C. A., Venable, J. C. & Snyder, S. H. (1987) *Soc. Neurosci. Abstr.* **13,** 362.