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Articles

Olfactory Transduction: Cross-Talk between Second-Messenger Systems†

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ABSTRACT: Chemosensory cilia of olfactory receptor neurons contain an adenylate cyclase which is stimulated by high concentrations of odorants. Cyclic AMP produced by this enzyme has been proposed to act as second messenger in olfactory transduction. Here we report that olfactory cilia contain calmodulin and that calmodulin potently activates olfactory adenylate cyclase by a mechanism additive to and independent from direct stimulation by odorants. Activation by calmodulin is calcium dependent and enhanced by GTP. Thus, olfactory transduction may involve a second-messenger cascade in which an odorant-induced increase in intracellular calcium concentration leads to activation of adenylate cyclase by calmodulin.

Olfaction takes place after air-borne odorants partition into the nasal mucus and interact with chemosensory cilia that protrude from the dendritic tips of olfactory receptor neurons (Getchell et al., 1984; Lancet, 1986; Anholt, 1989). Isolated olfactory cilia provide a preparation of chemosensory dendritic membranes thought to contain at least some, if not all, of the membrane-associated components that mediate odorant recognition and olfactory transduction (Anholt et al., 1986; Chen et al., 1986). Indeed, ciliary membrane preparations contain an odorant-sensitive adenylate cyclase (Pace et al., 1985; Sklar et al., 1986; Shirley et al., 1986; Pfeuffer et al., 1989) and its associated stimulatory GTP-binding protein (Pace et al., 1985; Anholt et al., 1987), G_{olf}, an olfactory neuron specific variant of G_s (Jones & Reed, 1989), and also odorant-gated cation channels (Labarca et al., 1988). The olfactory adenylate cyclase is stimulated by high concentrations of some, but not all, odorants (Pace et al., 1985; Sklar et al., 1986; Shirley et al., 1986), and cyclic AMP has, therefore, been proposed to act as second messenger in olfaction (Pace et al., 1985; Sklar et al., 1986; Shirley et al., 1986; Lancet & Pace, 1987; Nakamura & Gold, 1987; Gold & Nakamura, 1987). However, the enzyme is stimulated mostly by hydrophobic odorants at concentrations close to their aqueous solubility limits (Sklar et al., 1986; Anholt, 1987). Moreover, the same odorants at

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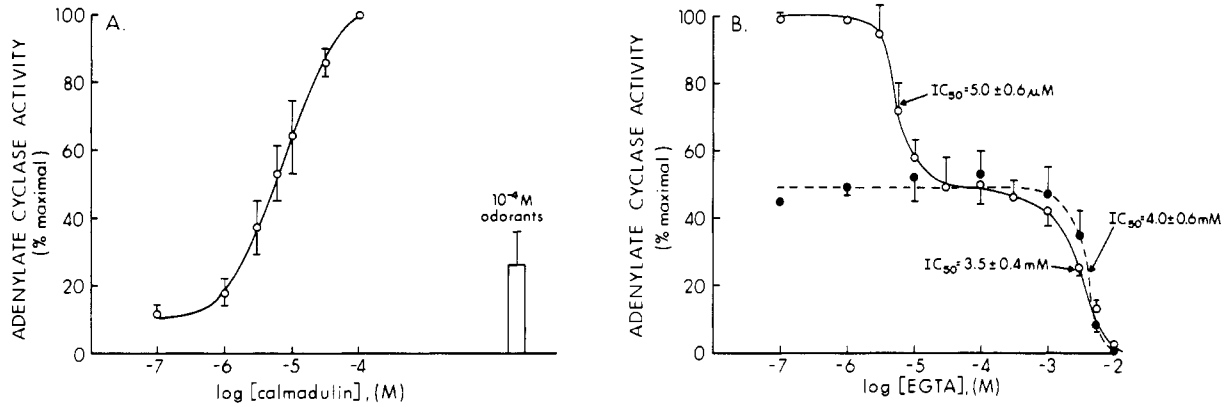

FIGURE 1: Stimulation of the olfactory adenylate cyclase by calcium/calmodulin. (A) Dose–response curve for stimulation of the olfactory adenylate cyclase by calmodulin. Calmodulin was added at the indicated concentrations in the presence of 100 μM GTP. Adenylate cyclase activities were corrected for the basal activity observed with GTP alone and standardized to the plateau level. The amplitude of the maximal response was obtained by subtracting the basal activity measured in the presence of GTP from the maximal calmodulin-stimulated activity. The maximal calmodulin-stimulated activity amounted to 3.17 ± 0.30 nmol min^{-1} (mg of protein)$^{-1}$ and was approximately 30–40% of the maximal activity observed in the presence of 10 μM forskolin. This is 3–6 times higher than the activity elicited by an odorant mixture of 3-isobutyl-2-methoxypyrazine, menthone, and citralva, each at 0.1 mM. Data points represent the averages of six independent experiments each consisting of triplicate measurements, and the error bars represent standard errors of the mean. (B) Dependence on calcium for adenylate cyclase activation by calmodulin. Adenylate cyclase activity was assayed in the presence of 100 μM GTP and the indicated concentrations of EGTA in the presence (open circles) or absence (closed circles) of 10 μM calmodulin. The amplitude of the maximal response was obtained by subtracting the basal activity measured in the absence of GTP from the maximal calmodulin-stimulated activity. Data points and error bars represent the averages and standard errors of the mean of six independent experiments each consisting of triplicate measurements. Note the biphasic appearance of the inhibition curve due to the removal of calcium at low concentrations and the removal of magnesium at high concentrations of EGTA. Calculated values for the approximate concentrations of endogenous calcium and magnesium at different concentrations of EGTA are listed in Table I.

similar concentrations increase cyclic AMP levels in melanocytes, which do not have an olfactory function (Lerner et al., 1988). Thus, the physiological significance of the effects of high concentrations of odorants on adenylate cyclase remains to be consolidated, and the role of cyclic AMP in olfaction has yet to be precisely defined.

Less attention has been paid to the role of calcium in olfactory transduction. Calcium at submicromolar concentrations modulates the activity of calcium-activated potassium channels (Maue & Dionne, 1987; Firestein & Werblin, 1987) and odorant-activated currents in isolated olfactory receptor cells (Kurahashi, 1989), calcium channel blockers inhibit the electroolfactogram (Winegar et al., 1988), and patch-clamp studies on isolated olfactory receptor cells have identified an inward calcium current (Trotier, 1986; Schild, 1989). These observations and the fact that calcium is known to activate brain adenylate cyclase via calmodulin (Brostrom et al., 1975, 1978; Westcott et al., 1979; Salter et al., 1981; Mollner & Pfeuffer, 1988; Uhlen & Wikberg, 1988) prompted us to investigate whether calcium could mediate olfactory transduction through activation of the olfactory adenylate cyclase.

Materials and Methods

Calmodulin (3′,5′-cyclic-nucleotide phosphodiesterase activator) and ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA)[1] were obtained from Sigma Chemical Co. (St. Louis, MO), forskolin was purchased from Calbiochem (La Jolla, CA), 3-isobutyl-2-methoxypyrazine was from Pyrazine Specialties (Atlanta, GA), and all nucleotides were from Boehringer Mannheim (Indianapolis, IN). Menthone and citralva (3,7-dimethyl-2,6-octadienenitrile) were generously donated by International Flavors and Fragrances, Inc. (Union Beach, NJ).

Olfactory cilia were detached from the olfactory epithelium of the bullfrog (*Rana catesbeiana*, purchased from Acadian Biological, Rayne, LA, and from Amphibians of North America, Nashville, TN) and subjected to sucrose gradient centrifugation, as described previously (Anholt et al., 1986). The ciliary membranes were resuspended in 2 mM HEPES, pH 7.4, 112 mM NaCl, 3.4 mM KCl, and 2.4 mM $NaHCO_3$. The protein concentration was measured by the method of Lowry et al. (1951) with bovine serum albumin as standard. The membranes were stored at −80 °C.

Adenylate cyclase activity was assayed by the method of Salomon (1979) at 20 or 40 μg/mL ciliary protein. The activity of 3′,5′-cyclic-nucleotide phosphodiesterase was measured by incubating olfactory cilia at 100 μg of ciliary protein/mL for 60 min at 30 °C in 50 μL of a reaction mixture containing 25 mM Tris–acetate, pH 8.0, 5 mM magnesium acetate, 50 μM cyclic AMP, and 2 μCi/mL [^{3}H]cAMP (Amersham, Arlington Heights, IL; specific radioactivity 34 Ci/mmol) in the presence or absence of 0.5 mM 3-isobutyl-1-methylxanthine (Aldrich Chemical Co., Milwaukee, WI). The reactions were stopped by addition of 100 μL of 2% sodium dodecyl sulfate/1.3 mM cyclic AMP and heating for 3 min at 90 °C, followed by ion-exchange chromatography according to Salomon (1979) to recover undegraded cyclic AMP. Reactions were run in triplicate, and phosphodiesterase activity was calculated as the 3-isobutyl-1-methylxanthine-inhibitable breakdown of cyclic AMP. All enzyme reactions were linear with time and protein concentration. Calmodulin concentrations in olfactory cilia were measured by Dr. Harry LeVine III at GLAXO, Inc. (Research Triangle Park, NC), using the calmodulin-binding assay described by LeVine et al. (1986).

Results

Endogenous calmodulin can readily be demonstrated in olfactory cilia preparations and amounts to 1.35 ± 0.36 (n = 5) μg of calmodulin/mg of protein. Calmodulin potently stimulates the olfactory adenylate cyclase (Figure 1A). This stimulation is half-maximal at 5.6 ± 0.9 μM calmodulin and saturates at about 30 μM calmodulin. Maximal activation

[1] Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; GDPβS, guanosine 5′-*O*-(2-thiodiphosphate).

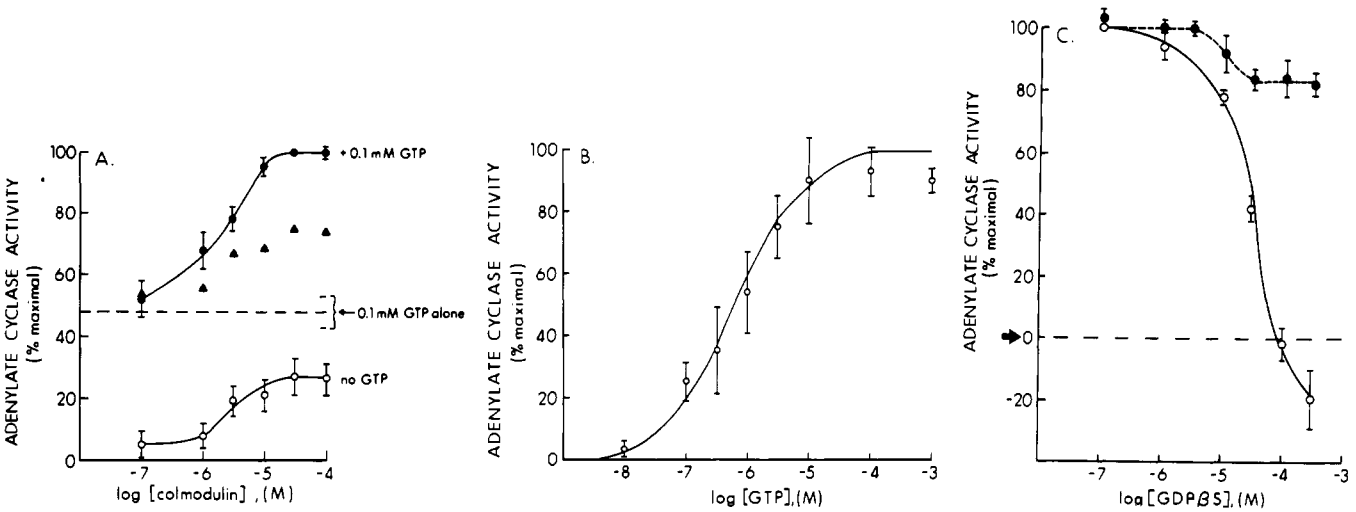

FIGURE 2: Enhancement by GTP of adenylate cyclase stimulation by calmodulin. (A) Stimulation of adenylate cyclase in the absence and presence of GTP. Adenylate cyclase activity was assayed as in Figure 1 at the indicated concentrations of calmodulin in the absence (open circles) or presence (closed circles) of 100 μM GTP. Data were corrected for the basal activity in the absence of GTP and standardized as described in the legend to Figure 1. The basal level of adenylate cyclase stimulation by GTP alone is indicated by the arrow and the dashed line and represents 48 ± 5% of the maximal activity. Maximal stimulation by calmodulin in the absence of GTP amounts to 26 ● 6% of the maximal stimulation observed in the presence of GTP. The triangles indicate values predicted for adenylate cyclase activation, if the sum of the activities elicited by GTP and calmodulin separately were identical with the activity elicited by both stimuli together. Data points and error bars represent the averages and standard errors of the mean of three experiments, each consisting of triplicate measurements. (B) Dose–response curve for the enhancement by GTP of adenylate cyclase stimulation by calmodulin. Adenylate cyclase activity was assayed at the indicated concentrations of GTP in the absence or presence of 10 μM calmodulin. Values obtained in the absence of calmodulin were subtracted from the corresponding values obtained in the presence of calmodulin, and the resulting values were calculated as percentages of the plateau value [1.25 ± 0.24 nmol min^{-1} (mg of protein)$^{-1}$]. Data points and error bars represent the averages and standard errors of the mean of five independent experiments, each consisting of triplicate measurements. (C) Inhibition of calmodulin-stimulated adenylate cyclase activity by GDPβS. Adenylate cyclase activity was assayed at the indicated concentrations of GDPβS in the presence of 10 μM calmodulin and 100 μM GTP (open circles) or 10 μM forskolin (closed circles). Values measured in the presence of calmodulin and GTP were corrected for the basal GTP-stimulated level (indicated by the arrow) and calculated as percentages of the maximal calmodulin- and GTP-induced activity in the absence of GDPβS. Note that inhibition by GDPβS extends below the basal GTP-stimulated level, reflecting inhibition of both the GTP-mediated activation of adenylate cyclase and the GTP-dependent component of the calmodulin-mediated activation. The concentration of GDPβS that corresponds to the midpoint between the plateau and the basal GTP-stimulated level is 28 ± 2 μM. Calmodulin activation observed in the absence of GTP (panel A, open circles) is not sensitive to GDPβS. Values measured in the presence of forskolin were corrected for the basal activity in the absence of GTP and calculated as percentages of the maximal forskolin-stimulated activity in the absence of GDPβS. Data points and error bars represent the averages and standard errors of the mean for the calmodulin- and forskolin-stimulated activities of four and three independent experiments, respectively, each consisting of triplicate measurements.

of the enzyme by calmodulin is 3–6 times greater than that observed with a mixture of odorants.

Activation of the olfactory adenylate cyclase by calmodulin is dependent on endogenous calcium and is eliminated when calcium is removed by EGTA (Figure 1B). Inhibition by EGTA of calcium/calmodulin-activated adenylate cyclase activity is biphasic with midpoints at 5.0 ± 0.6 μM and 4.0 ± 0.6 mM EGTA. The lower midpoint reflects chelation of calcium and resulting inhibition of calmodulin stimulation, whereas the higher midpoint reflects the removal of magnesium which results in further suppression of the GTP-stimulated basal level (Figure 1B; Table I). Estimates of the concentrations of free calcium at different concentrations of EGTA show that an increase in calcium concentration from approximately 0.1 μM to approximately 2 μM will lead to calcium/calmodulin-mediated activation of the olfactory adenylate cyclase (Table I).

The mechanism of activation of the olfactory adenylate cyclase by calcium/calmodulin involves a GTP-dependent and a GTP-independent component. Stimulation of the enzyme by calmodulin in the absence of added GTP represents approximately 26% of that observed in the presence of GTP (Figure 2A, open circles and closed circles, respectively). Under our experimental conditions, GTP alone elicited significant stimulation over the basal activity. However, stimulation observed by GTP and calmodulin together was synergistic and resulted in approximately 27% greater adenylate cyclase activity than the activity predicted from summation of the GTP-mediated activation and stimulation by calmodulin measured separately (Figure 2A, triangles). This GTP-dependent component of the activation by calmodulin is further documented in Figure 2B, which shows its dependence on the concentration of GTP. The midpoint of this dose–response curve corresponds to 600 ± 140 nM GTP. Both the stimulation of adenylate cyclase by GTP alone and the GTP-dependent component of calcium/calmodulin-induced adenylate

Table I: Estimated Free Concentrations of Divalent Cations in the Presence of Various Concentrations of EGTA during Stimulation of the Olfactory Adenylate Cyclase by 10 μM Calmodulin[a]

[EGTA] (M)	[free Ca^{2+}] (μM)	[free Mg^{2+}] (mM)
10^{-6}	2.2	4
5×10^{-6}	0.78	4
10^{-5}	0.15	4
10^{-3}	0	3.2
3.5×10^{-3}	0	1.6
10^{-2}	0	0.4

[a] Stability constants of EGTA for H^+, Ca^{2+}, and Mg^{2+} were corrected for pH (8.0), temperature (30 °C), and ionic strength (0.15 M monovalent salt) according to Robertson and Potter (1984). Stability constants of 1.584×10^7 M^{-1} for H^+, 7.58×10^6 M^{-1} for Ca^{2+}, and 85.7 M^{-1} for Mg^{2+} were used to calculate the concentrations of free Ca^{2+} and Mg^{2+} using a computer program (Fabiato & Fabiato, 1979) and assuming an affinity constant for calcium of 1.0 μM for calmodulin and an estimated total endogenous calcium concentration of 10 μM. Inhibition of adenylate cyclase becomes apparent at this calcium concentration (Sklar et al., 1986; Shirley et al., 1986; Seamon & Daly, 1986), and we know empirically that calcium concentrations under our conditions fall just short of this inhibitory concentration. The total magnesium concentration (after allowing for binding to ATP) is 4 mM (see also Figure 1B).

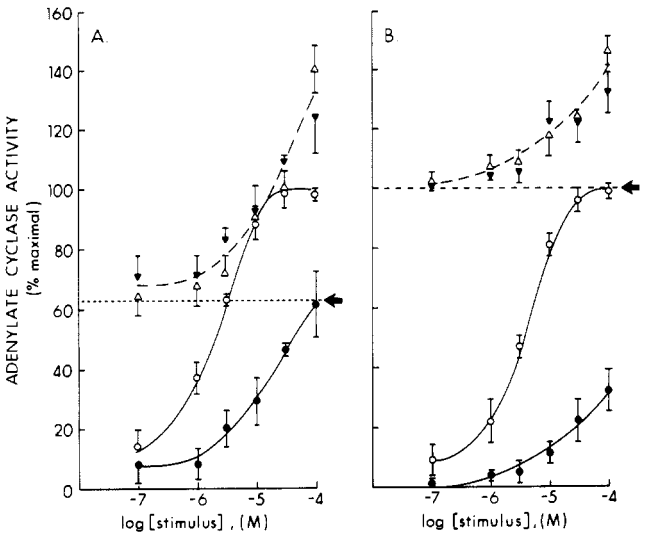

FIGURE 3: Additive stimulation of olfactory adenylate cyclase by odorants and calmodulin. (A) Additive stimulation of adenylate cyclase by odorants and 3 μM calmodulin. Adenylate cyclase activity was assayed in the presence of 100 μM GTP at the indicated concentrations of calmodulin (open circles) or an equimolar mixture of the odorants, 3-isobutyl-2-methoxypyrazine, menthone, and citralva (closed circles), and in the presence of odorants at the indicated concentrations plus a submaximal level of 3 μM calmodulin (open triangles). Values were corrected for the basal GTP-stimulated activity and calculated as percentages of the activity measured at the calmodulin-stimulated plateau. The arrow and the dashed line indicate the activity elicited at 3 μM calmodulin. Predicted values for additivity between odorant stimulation and stimulation by 3 μM calmodulin are indicated by the closed triangles. Note that dose–response curves to odorants do not saturate due to limitations on their aqueous solubilities, as reported previously (Pace et al., 1985; Sklar et al., 1986; Shirley et al., 1986). Activation of adenylate cyclase by odorants was GTP-dependent, as reported previously (Pace et al., 1985; Sklar et al., 1986). The data points and error bars represent the averages and standard errors of the mean of three experiments, each consisting of triplicate measurements. (B) Additive stimulation of adenylate cyclase by odorants and 100 μM calmodulin. The conditions were identical with those in panel A, except that a saturating concentration of 100 μM rather than 3 μM calmodulin was used to assess additivity between odorant activation and stimulation by calmodulin of the olfactory adenylate cyclase. The arrow and dashed line indicate the activity elicited by 100 μM calmodulin. The data points and error bars represent the averages and standard errors of the mean of four independent experiments, each consisting of triplicate measurements.

cyclase activation can be inhibited by GDPβS (Figure 2C, open circles). GDPβS has a relatively small (about 17%), but reproducible, inhibitory action on the forskolin-stimulated activity, perhaps reflecting involvement of the stimulatory G-protein in the formation of a high-affinity forskolin-binding site (Figure 2C, closed circles; Seamon & Daly, 1986). Activation of the olfactory adenylate cyclase by calmodulin in the absence of GTP is not affected by GDPβS (data not shown).

We investigated whether stimulation of the olfactory adenylate cyclase by odorants and by calmodulin would be synergistic or additive. In contrast to the synergism observed between adenylate cyclase stimulation by calmodulin and GTP, described above, activation by odorants and calmodulin is additive when the dose–response to a mixture of odorants is measured in the absence and presence of a conditioning concentration of calmodulin both at submaximal and at saturating concentrations of calmodulin (Figure 3A,B). Thus, odorants at high micromolar concentrations and calmodulin activate the olfactory adenylate cyclase via independent mechanisms.

Measurements of 3′,5′-cyclic-nucleotide phosphodiesterase activity in our ciliary membrane preparations reveal an activity of 2.32 ± 0.15 nmol min^{-1} (mg of protein)$^{-1}$ (n = 6). We did not detect stimulation of this phosphodiesterase activity by calmodulin up to a concentration of 100 μM.

Discussion

We have demonstrated that the adenylate cyclase present on chemosensory cilia of olfactory receptor neurons is stimulated by calcium/calmodulin. Endogenous calmodulin amounts to 1.35 ± 0.36 μg of calmodulin/mg of ciliary protein. Some calmodulin may be lost from the ciliary membranes during the isolation procedure, since preparing the cilia involves incubation of the epithelium in a solution containing EDTA followed by detachment of the cilia via a calcium shock (Anholt et al., 1986). Thus, measurements of calmodulin in these preparations are minimal estimates of the concentrations of calmodulin in vivo. The measured concentration of endogenous calmodulin represents approximately 0.1% of the total ciliary protein and is comparable to the high levels of calmodulin found in brain, which reflect concentrations in the 10^{-5} M range (Egrie et al., 1977; Klee et al., 1980), consistent with the range of concentrations over which calmodulin activates the olfactory adenylate cyclase in our experiments. The concentration of endogenous calmodulin in our assays is around 1 μM, the threshold concentration for activation of adenylate cyclase (Figure 1A). Thus, the actual EC_{50} for activation of adenylate cyclase by calmodulin may be 15–20% lower than apparent from the dose–response curve in Figure 1A.

Stimulation of the olfactory adenylate cyclase by calmodulin and by GTP is synergistic. Previous studies on calcium/calmodulin-regulated adenylate cyclase from brain have demonstrated potentiation by calmodulin of stimulation by GTP (Brostrom et al., 1978; Mickevicius et al., 1986). These studies showed that calmodulin increases the potency with which guanine nucleotides activate adenylate cyclase without altering the maximal activity of the enzyme. In contrast, our study shows that, in the case of the olfactory adenylate cyclase, GTP potentiates calmodulin-mediated activation by an increase in the total cyclic AMP production rather than a reduction in the EC_{50} for calmodulin (Figure 2A).

In contrast to the synergism observed between GTP and calmodulin, activation of the enzyme by odorants and calmodulin in the presence of GTP is additive, indicating that odorants and calmodulin activate adenylate cyclase via independent mechanisms (Figure 3).

To further characterize calmodulin-mediated activation of the olfactory adenylate cyclase, we tested the effect of the calmodulin antagonist calmidazolium. Calmidazolium inhibited calmodulin-stimulated activity with an IC_{50} of 50 ± 7 μM (n = 4). However, the specificity of this inhibition is difficult to assess, since calmidazolium inhibited forskolin-activated enzyme activity in a similar manner (data not shown). Inhibition of the electroolfactogram by the calmodulin antagonists trifluoperazine, chlorpromazine, and *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W-7) has been observed, but these effects were hard to interpret, since in these experiments the inhibitory potencies of these compounds did not correlate with their potencies as calmodulin antagonists, possibly due to nonspecific membrane effects (Winegar et al., 1988).

Previous models of olfactory transduction have focused on activation of adenylate cyclase by odorants without considering a role for calcium (Lancet & Pace, 1987; Gold & Nakamura, 1987). Our demonstration that calcium at physiological concentrations activates this enzyme through calmodulin via a GTP-dependent and a GTP-independent mechanism suggests a role for calcium in regulation of the olfactory adenylate

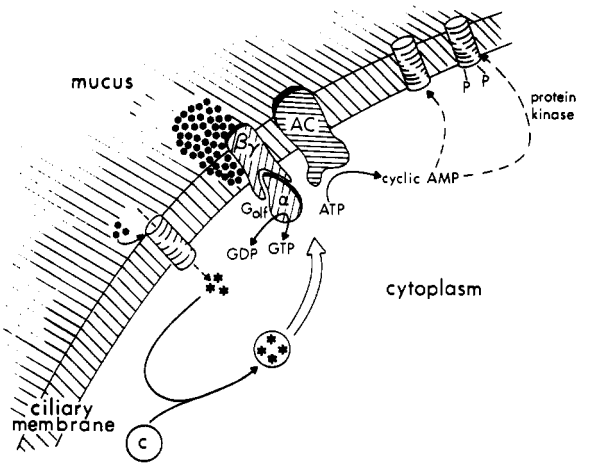

FIGURE 4: Hypothetical model for olfactory transduction. Activation of olfactory receptor cells by odorants (indicated by the hexagons) may be initiated via the influx of calcium (indicated by the stars; Trotier, 1986; Winegar et al., 1988; Schild, 1989), possibly through odorant-gated cation channels (Labarca et al., 1988) or as a result of other transduction processes such as phosphatidylinositol hydrolysis. Subsequent formation of a calcium/calmodulin complex (shown as a circle containing four stars; c designates calmodulin) is followed by activation of the olfactory adenylate cyclase. The resulting cyclic AMP would then signal activation of the cell via protein phosphorylation (Heldman & Lancet, 1986) or direct gating of ion channels (Nakamura & Gold, 1987). When odorants reach high micromolar concentrations, the calcium pathway can be bypassed and the enzyme can be directly activated via its stimulatory G-protein, G_{olf} (Jones & Reed, 1989), perhaps as a result of partitioning of odorants in the membrane (Anholt, 1987). The precise nature of odorant recognition sites, permeability properties of odorant-activated channels, and identity of substrates for cyclic AMP dependent protein kinase remain to be determined. It should also be noted that calcium/calmodulin may affect cellular targets other than adenylate cyclase and that the precise function of cyclic AMP in olfactory transduction has not been conclusively determined. The speculative nature of this working hypothesis should, therefore, be emphasized, and extensive revisions of this model can be anticipated as new information becomes available.

cyclase and underscores the importance of cross-talk between second-messenger systems in olfactory transduction. We suggest that odorants at physiological concentrations may cause mobilization of calcium (Trotier, 1986; Winegar et al., 1988) which then leads to a rise in intracellular cyclic AMP levels via the formation of a calcium/calmodulin complex (Figure 4). Only at high micromolar concentrations of odorants would the calcium pathway be bypassed by direct activation of the olfactory adenylate cyclase (Figure 4; Pace et al., 1985; Sklar et al., 1986; Shirley et al., 1986).

It should be noted that calcium/calmodulin may also act on other cellular targets that could affect olfactory transduction, such as 3′,5′-cyclic-nucleotide phosphodiesterase [reviewed by Klee et al. (1980)]. Phosphodiesterase-mediated hydrolysis of cyclic AMP in our olfactory cilia preparations amounted to 2.32 ± 0.15 nmol min^{-1} (mg of protein)$^{-1}$ and is similar to the basal rate of cyclic AMP formation via adenylate cyclase (Sklar et al., 1986; Anholt, 1987). Although we did not detect stimulation by calcium/calmodulin of our membrane-associated phosphodiesterase activity, we cannot exclude the possible loss of a soluble calmodulin-sensitive cytoplasmic form of phosphodiesterase during the preparation of olfactory cilia.

Olfactory receptor neurons possess a high membrane impedance (Trotier, 1986; Maue & Dionne, 1987; Firestein & Werblin, 1987). These cells are electrotonically compact, and only picoamperes of injected current are sufficient to elicit action potentials (Maue & Dionne, 1987; Firestein & Werblin, 1987; Hedlund et al., 1987). Consequently, tight regulation of the membrane potential at rest and during activation by odorants is essential for these highly sensitive cells. the second-messenger cascade which we propose would provide at the same time signal amplification and multiple points of control over the cellular response to odorants.

ACKNOWLEDGMENTS

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Registry No. 5-GTP, 86-01-1; GDPβS, 71376-97-1; Ca, 7440-70-2; Mg, 7439-95-4; adenylate cyclase, 9012-42-4; 3′,5′-cyclic nucleotide phosphodiesterase, 9040-59-9.

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Effect of Erythrocyte Transbilayer Phospholipid Distribution on Fusion with Vesicular Stomatitis Virus

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ABSTRACT: To identify the specific component(s) in the target membrane involved in fusion of vesicular stomatitis virus (VSV), we examined the interaction of the virus with human erythrocyte membranes with asymmetric and symmetric bilayer distributions of phospholipids. Fusion was monitored spectrofluorometrically by the octadecylrhodamine dequenching assay. Fusion of VSV with lipid-symmetric erythrocyte ghosts was rapid at 37 °C and low pH, whereas little or no fusion was observed with lipid-asymmetric ghosts. Conversion of phosphatidylserine in the lipid-symmetric ghost membrane to phosphatidylethanolamine by means of the enzyme phosphatidylserine decarboxylase did not alter the target membrane's susceptibility to VSV fusion. Spin-labeled phospholipid analogues with phosphatidylserine, phosphatidylethanolamine, and phosphatidylcholine headgroups incorporated into the outer leaflet of lipid-asymmetric erythrocytes did not render those membranes fusogenic. Electron spin resonance spectra showed an increased mobility of a phosphatidylcholine spin-label incorporated into the outer leaflet of lipid-symmetric erythrocyte ghosts as compared to that of lipid-asymmetric ghosts. These results indicate that the susceptibility to VSV fusion is not dependent on any particular phospholipid but rather is related to packing characteristics of the target membrane.

The envelope of vesicular stomatitis virus (VSV)[1] consists of a bilayer membrane with a single type of spike glycoprotein, the G protein, which mediates attachment to the cell surface and induces fusion between viral and target membranes (Pal et al., 1987). pH-dependent fusion of VSV with cells has been studied by a variety of methods (White et al., 1981; Matlin et al., 1983; Yamada & Ohnishi, 1986; Blumenthal et al., 1987). However, it is not clear what components are necessary in target membranes to render them susceptible to VSV fusion. On the basis of inhibition studies, Schlegel et al. (1983) and Mastromarino et al. (1988) support the notion of phospholipid or sialoglycolipid specificity for VSV penetration. On the other hand in studies of fusion of VSV with liposomes Yamada and Ohnishi (1986) showed no phospholipid specificity.

We examine the issue of target membrane specificity using the octadecylrhodamine (R18) assay (Hoekstra et al., 1984), which directly monitors fusion between the virus and a biological membrane. As the biological target we chose the erythrocyte membrane, whose phospholipid arrangement can readily be modified. The phospholipids of normal erythrocytes are arranged asymmetrically across the plasma membrane; phosphatidylcholine (PC) and sphingomyelin are predomiantly on the outer surface, whereas others such as phosphatidylserine (PS) and phosphatidylethanolamine (PE) are predominantly restricted to the inner leaflet (Zwaal et al., 1975). However, erythrocytes can be lysed and resealed under conditions where the asymmetric distribution of phospholipids is lost or retained (Williamson et al., 1985). We have recently shown that

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[1] Abbreviations: VSV, vesicular stomatitis virus; R18, octadecylrhodamine B chloride; PBS, phosphate-buffered saline; ESR, electron spin resonance; PC, phosphatidylcholine; PS, phosphatidylserine; PE, phosphatidylethanolamine; (0,2)PC, 1-palmitoyl-2-(4-doxylpentanoyl)-PC; (0,2)PS, 1-palmitoyl-2-(4-doxylpentanoyl)-PS; (0,2)PE, 1-palmitoyl-2-(4-doxylpentanoyl)-PE; PSD, PS decarboxylase; FDQ, fluorescence dequenching; RBC, red blood cell(s).