The Journal of Neuroscience
July 1986, 6(7): 1962-1969

A Partially Purified Preparation of Isolated Chemosensory Cilia from the Olfactory Epithelium of the Bullfrog, *Rana catesbeiana*

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Cilia at the tips of dendritic processes of olfactory receptor cells are the sites of initial recognition and transduction events in olfactory reception. We have detached cilia from the olfactory epithelium of the bullfrog, *Rana catesbeiana,* via a calcium shock and partially purified them in high yield (226 ± 19 μg protein/frog, n = 14) by sucrose gradient centrifugation. The cilia appear to undergo osmotic lysis during the isolation procedure, forming isolated axonemal structures and ciliary plasma membrane vesicles with diameters of 100-500 nm and an internal volume of 2.3 ± 0.5 μl/mg protein. PAGE in SDS reveals approximately 30 protein bands, among which cytoskeletal components, such as tubulin and actin, are readily identifiable by immunoblotting. Approximately 15 glycoprotein bands reactive with concanavalin A are discernible with major glycopeptides at apparent molecular weights of 56-65, 95, and 116 kDa. In contrast to olfactory cilia, respiratory cilia, isolated from the palate of the frog, do not contain the prominent glycopeptides observed for olfactory cilia. The 56-65 kDa glycopeptide region reacts with antiserum against chick kidney, Na^+/K^+-ATPase, and contains the β subunit of this enzyme. In addition, we have identified the α and β subunits of a quanine nucleotide-binding protein (G-protein) in the olfactory cilia preparation. This preparation of isolated olfactory cilia from *Rana catesbeiana* represents a readily accessible model system for studies of initial events in chemosensory recognition and signal transduction in the olfactory system.

Olfactory reception in vertebrates is mediated via receptor cells located in the olfactory epithelium. These cells are bipolar neurons. They project an axon across the cribriform plate of the ethmoid bone into the glomerular layer of the olfactory bulb, where the first synaptic relay occurs. In addition, they extend a dendrite towards the nasal lumen (for reviews, see Getchell et al., 1985, and Lancet, 1984, 1986). This dendrite carries at its apex a group of cilia that are in contact with the nasal mucus (Getchell et al., 1985; Lancet, 1984, 1986; Menco, 1980; Reese, 1965). The cilia appear to be the site where the initial chemosensory recognition and transduction events take place. This notion is based partly on analogies with sensory organelles of the visual and auditory systems and is supported by several lines of evidence. Lavage of the olfactory epithelium of the frog with Triton X-100 removes the cilia while leaving the rest of the receptor cells intact. This results in the abolition of the electro-olfactogram, a complex electrical signal observed after odor application. The return of the electro-olfactogram is correlated with the reappearance of olfactory cilia (Adamek et al., 1984). Electron-microscopic observations of freeze-fracture replicas reveal numerous particles in membranes of cilia from olfactory epithelium, while only few particles are detected in cilia from respiratory epithelium (Menco, 1980; Menco et al., 1976). In addition, Rhein and Cagan (1980) have described binding sites for odorant amino acids on cilia isolated from the olfactory rosette of the rainbow trout.

Techniques for the detachment of cilia were initially developed for studies on protozoa (Adoutte et al., 1980), such as paramecium and tetrahymena (Gibbons, 1965; Watson and Hopkins, 1962) and lamellibranch mollusks (Linck, 1973). Based on these studies, Rhein and Cagan (1980) applied a calcium shock to detach and isolate cilia from the olfactory organ of the rainbow trout. Subsequently, Chen and Lancet (1984) reported the isolation of olfactory cilia from the frog, *Rana ridibunda,* using a similar method. The frog is eminently suitable for morphological, electrophysiological, and developmental studies of the olfactory system. The olfactory and respiratory epithelia are well separated. The olfactory epithelium is readily accessible and consists of a dorsal and a ventral sheet that can easily be dissected. The olfactory cilia have been reported to reach lengths of up to 200 μm (Reese, 1965) and can be readily detached from the epithelium.

We shall describe a procedure by which a preparation enriched in olfactory cilia can be obtained from the bullfrog, *Rana catesbeiana.* The bullfrog yields up to 7 times more material per animal than the smaller frogs, such as *Rana ridibunda* and *Rana pipiens.* We also describe some of the major ultrastructural and biochemical characteristics of this preparation.

Materials and Methods

Isolation of olfactory cilia

Rana catesbeiana (20–25 cm) were supplied by Amphibians of North America (Nashville, TN) or Acadian Biological (Rayne, LA). *Rana pipiens* were obtained from Nasco (Ft. Atkinson, WI). The animals were maintained in a well-ventilated facility in a tank with circulating water. Frogs were killed by decapitation, and the ventral and dorsal sheets of olfactory epithelia were removed from both nasal chambers. The palate was dissected for the preparation of respiratory cilia. The tissues were bathed for 20 min in ice-cold Ringer's solution supplemented with 2 mM EDTA to dissolve the mucus. During this incubation period the solution was replaced twice with fresh solution. Subsequently, the medium was replaced with ice-cold Ringer's solution supplemented with 10 mM $CaCl_2$, and the tissue gently agitated on an end-over-end shaker for 10 min at 4°C. The deciliated epithelia were removed by centrifugation for 2 min at medium speed in a Clay-Adams Dynac serological centrifuge. The supernatant containing the detached cilia was centri-

Received June 27, 1985; revised Oct. 14, 1985; accepted Dec. 30, 1985.

This work was supported by a grant from International Flavors and Fragrances, Inc. U.A. is supported by N.I.H. Grant GM-31940 and is the recipient of a research award from the Maurice Müller Foundation of Switzerland. We thank Drs. Pamela Sklar and Doron Lancet for valuable discussions and Dawn C. Dodson for secretarial assistance. We are especially grateful to Naomi Taylor for excellent technical assistance, and to Dr. Eva Neer and Drs. Tamkun and Fambrough for the donation of antisera.

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fuged once again and the resulting supernatant (usually 3 ml, containing cilia from one frog/ml) was layered on top of 2 ml of 45% (wt/wt) sucrose in Ringer's solution. The gradients were centrifuged at 4°C for 30 min at 350,000 × *g* in a Beckman SW55Ti rotor. The cilia were collected from the interface on top of the sucrose cushion, diluted in an equal volume of Ringer's solution, and pelleted by centrifugation at 4°C for 15 min at 350,000 × *g* in the SW55Ti rotor. The pellet was resuspended in a small volume of Ringer's solution containing 2 mM EGTA. The protein concentration of the suspension was measured according to the method of Lowry et al. (1951), using BSA as standard. Cytochrome oxidase activity, used as an index of contamination, was assayed by a polarographic method, as described previously (Anholt et al., 1986). The concentrated suspension of isolated cilia could be stored at −70°C.

Measurement of apparent internal volume

The apparent internal volume of the cilia preparation was measured by incubation of an aliquot of the cilia suspension with 0.5–1.0 μCi of $^{86}Rb^+$ (New England Nuclear, Boston, MA) for 48 hr at 4°C. Internal $^{86}Rb^+$ was separated from the external radioisotope, as described by Gasko et al. (1976), by passage through a Dowex 50W8X cation exchange resin equilibrated in the Tris form. Assays were performed in triplicate and the column eluates counted with a scintillation counter after addition of Formula 963 scintillation cocktail (New England Nuclear, Boston, MA).

Electron microscopy

For electron-microscopic examination, olfactory cilia were pelleted by centrifugation and the pellets fixed for 3 hr with 2.5% glutaraldehyde (Electron Microscopy Sciences, Ft. Washington, PA) in Ringer's solution containing 1 mM $CaCl_2$. After overnight incubation in ice-cold buffer, the pellets were postfixed for 1 hr with ice-cold 1% OsO_4 (Electron Microscopy Sciences), dehydrated through a graded series of ethanol, and embedded in Araldite 6005 (Electron Microscopy Sciences). Sections (80 nm) were cut on a Sorvall Porter-Blum MT2B ultramicrotome and the specimens were examined under a Zeiss EM9S-2 electron microscope at 60 kV.

For negative staining, samples (~3 μl) were placed on carbon-coated 400-mesh/in. copper grids, which were briefly blotted with a drop of Ringer's solution to render them wet. After 60 sec, the grids were blotted with filter paper and washed for 30 sec upside-down on several drops of Ringer's solution and subsequently water. Finally, they were negatively stained for 30 sec upside-down on several drops of 0.75% uranyl formate, pH 4.25. Before the grids were allowed to dry, excess stain was blotted with filter paper and further drained off by gentle suction with a capillary.

Specimens were examined in a Zeiss EM10C transmission electron microscope operated at an acceleration voltage of 80 kV. Electron micrographs were recorded at either 5000 or 25,000× nominal magnification on Kodak SO-163 electron image film and developed for 4 min in 3× diluted Kodak D-19 developer. Magnification was calibrated using negatively stained catalase crystals, as described by Wrigley (1968).

PAGE and electrophoretic transfer

PAGE in SDS was performed after treatment of the samples with 2-mercaptoethanol on 10% slab gels in the discontinuous buffer system of Laemmli (1970). Protein bands were visualized by the silver-stain method of Wray et al. (1981). Myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase B (97.4 kDa), BSA (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), and trypsin inhibitor (20.1 kDa), all obtained from Sigma, were used as molecular weight markers. Electrophoretic transfer of proteins onto a nitrocellulose membrane (Schleicher and Schuell, Keene, NH) was performed at 10°C for 2½ hr at 80 V in a TE50 Transphor unit (Hoefer Scientific Instruments, San Francisco, CA) in 25 mM Tris, 192 mM glycine, 20% (vol/vol) methanol, pH 8.3. After transfer, the gel was silver-stained to ascertain that the transfer of proteins was complete. The membrane was stained with amido black or stored at 4°C in 50 mM Tris-HCl, 1 mM EDTA, 0.1% gelatin, 0.1% Triton X-100, pH 7.7.

Immunoblotting

Monoclonal antibodies against α and β tubulin from bovine brain were generously donated by Dr. Steve Blose (Cold Spring Harbor Laboratories, Long Island, NY). Monoclonal anti-actin was obtained from Amersham (Arlington Heights, IL). Rabbit antisera against the α and β subunits of a G-protein purified from cortex of rat brain were generously provided by Dr. Eva Neer (Brigham and Women's Hospitals, Boston, MA) and are described by Huff et al. (1985). Rabbit antiserum against the Na^+/K^+-ATPase purified from chick kidney was donated by Drs. Michael Tamkun and Douglas Fambrough (Carnegie Institute of Washington, Baltimore, MD) and is described by Tamkun and Fambrough (1986).

For immunoblotting, nitrocellulose blots were cut into narrow strips which were incubated for 1 hr at room temperature in 10 mM sodium phosphate buffer, pH 7.5, 0.9% NaCl, 0.05% Triton X-100, containing the desired antiserum at the appropriate dilution. Bound antibody was detected using the Vectastain kit from Vector Laboratories (Burlingame, CA). The reaction is based on the formation of complexes of biotinylated secondary antibody, avidin, and biotinylated HRP. The complexes were visualized in 10 mM sodium phosphate, pH 7.5, 0.9% NaCl, containing 0.5 mg/ml 3,3′-diaminobenzidine-4 HCl (Sigma) and 0.015% hydrogen peroxide.

Results

Preparation of isolated olfactory cilia

Prior to the isolation of chemosensory cilia from the olfactory epithelium of the bullfrog, the dissected tissue is incubated in the presence of EDTA to remove the mucus. The cilia are then detached by the addition of an excess of calcium ions. After removal of the deciliated epithelia, the cilia can be collected from the medium by centrifugation. Electron micrographs of this preparation show numerous ciliary structures, which, in cross section, display the characteristic 9 + 2 axonemal core (Fig. 1*A*, arrows). In addition, some contaminating material is sometimes observed, which at higher magnification is seen to contain pigment granules and appears, therefore, to be derived largely from melanocytes in the base of the lamina propria (Fig. 1*A*, arrowheads). These contaminants sediment as a black pellet after centrifugation on a 45% (wt/wt) sucrose cushion, leaving a white band of cilia at the interface with the sucrose cushion. The cilia are diluted with Ringer's solution and collected by centrifugation. They appear to be highly fragile and most of them undergo lysis during the isolation procedure. Electron micrographs reveal axonemes mostly devoid of a surrounding plasma membrane, as well as numerous membrane vesicles ranging from 100 to 500 nm in diameter (Fig. 1*B*). Many of these vesicles appear to be derived from ciliary plasma membranes, as is evident from examination of the preparation after negative staining (Fig. 2). Electron micrographs of negatively stained preparations reveal axonemal structures that, along their entire lengths, appear to be associated with numerous membrane fragments that adhere to or detach from the axonemal cores, forming isolated membrane vesicles (Fig. 2). The negatively stained cilia have a bipolar appearance. One end frequently appears branched, revealing splits along the length of the cilium, as was previously described by Reese (1965), and probably corresponds to the distal end. The other end may represent the detachment site near the ciliary necklace (Fig. 2*a*). Equilibration of the preparation for 48 hr with $^{86}Rb^+$ indicates an internal volume of 2.3 ± 0.5 μl/mg protein (n = 4). Cytochrome oxidase activity, indicative of mitochondrial contamination, amounts to 6.5 ± 0.2 nanoatoms of oxygen/min/mg protein (n = 3). It is difficult to obtain a precise measurement for the activity of cytochrome oxidase in homogenates from whole olfactory epithelium because of the presence of a large amount of pigmented particulate debris, which appears to interfere with the assay. However, we estimate the specific activity of the enzyme in the whole epithelium to be at least 10-fold higher than the specific activity measured for the isolated cilia. The yield of olfactory cilia obtained from *Rana catesbeiana* amounts to 226 ± 19 μg protein per frog (n = 14) and is about 7× higher than the yield of cilia obtained from *Rana pipiens* (32 ± 10 μg/ml protein; n = 6), the American counterpart of the Israeli frog, *Rana ridibunda,*

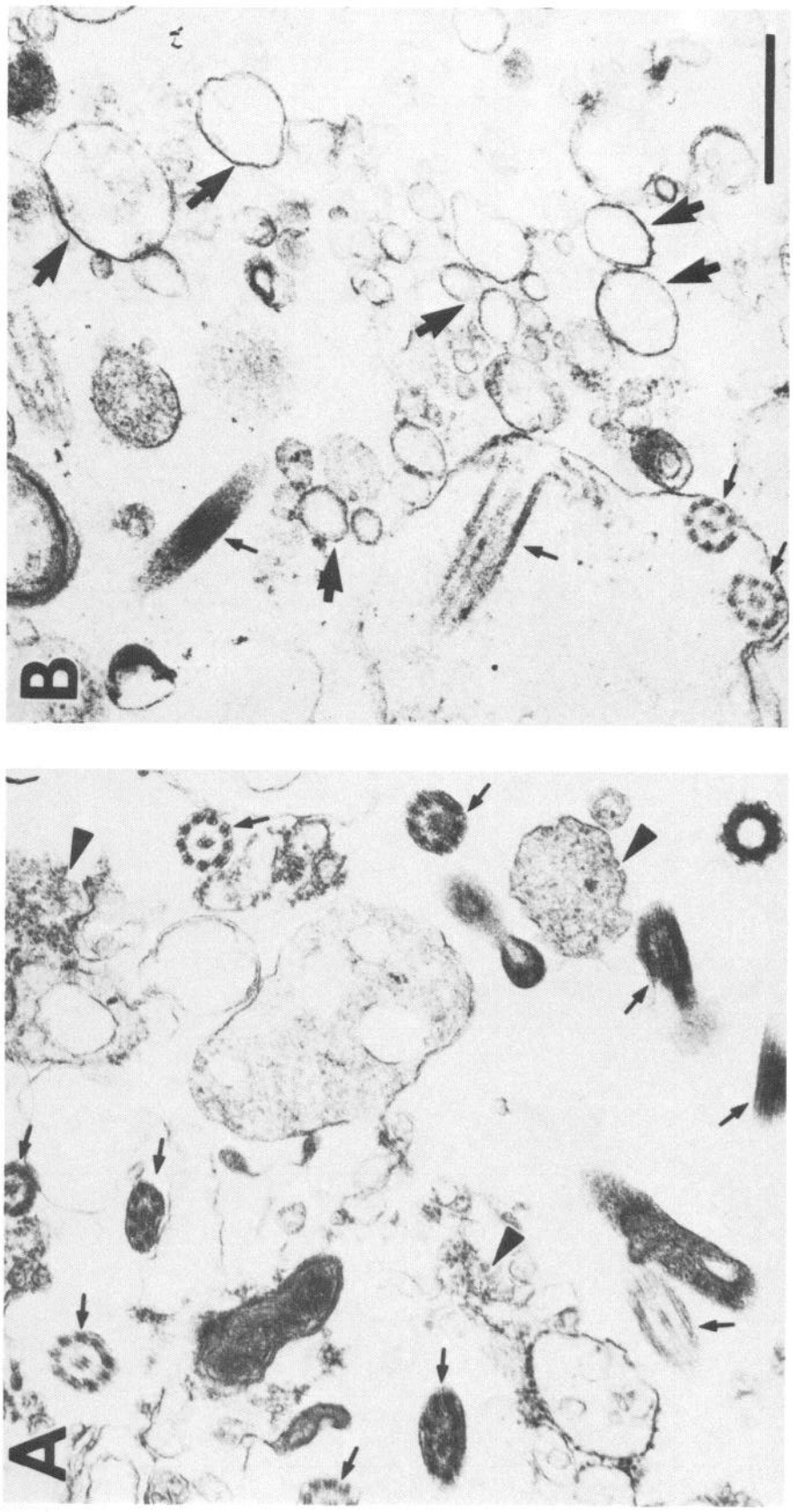

Figure 1. Electron micrographs of olfactory cilia detached from the olfactory epithelium of *Rana catesbeiana*. *A*, Olfactory cilia immediately after detachment from the epithelium. Numerous cilia in various orientations can be identified (*small arrows*). The *arrowheads* indicate contaminants, which appear to contain pigment granules and are presumably derived from melanocytes near the base of the lamina propria. *B*, Olfactory cilia after isolation on a 45% (wt/wt) sucrose cushion. Most of the cilia appear to have undergone osmotic lysis. Axonemal structures devoid of a surrounding membrane can be observed (*small arrows*) along with numerous membrane vesicles ranging between 100 and 500 nm in diameter (*large arrows*). Bar, 500 nm.

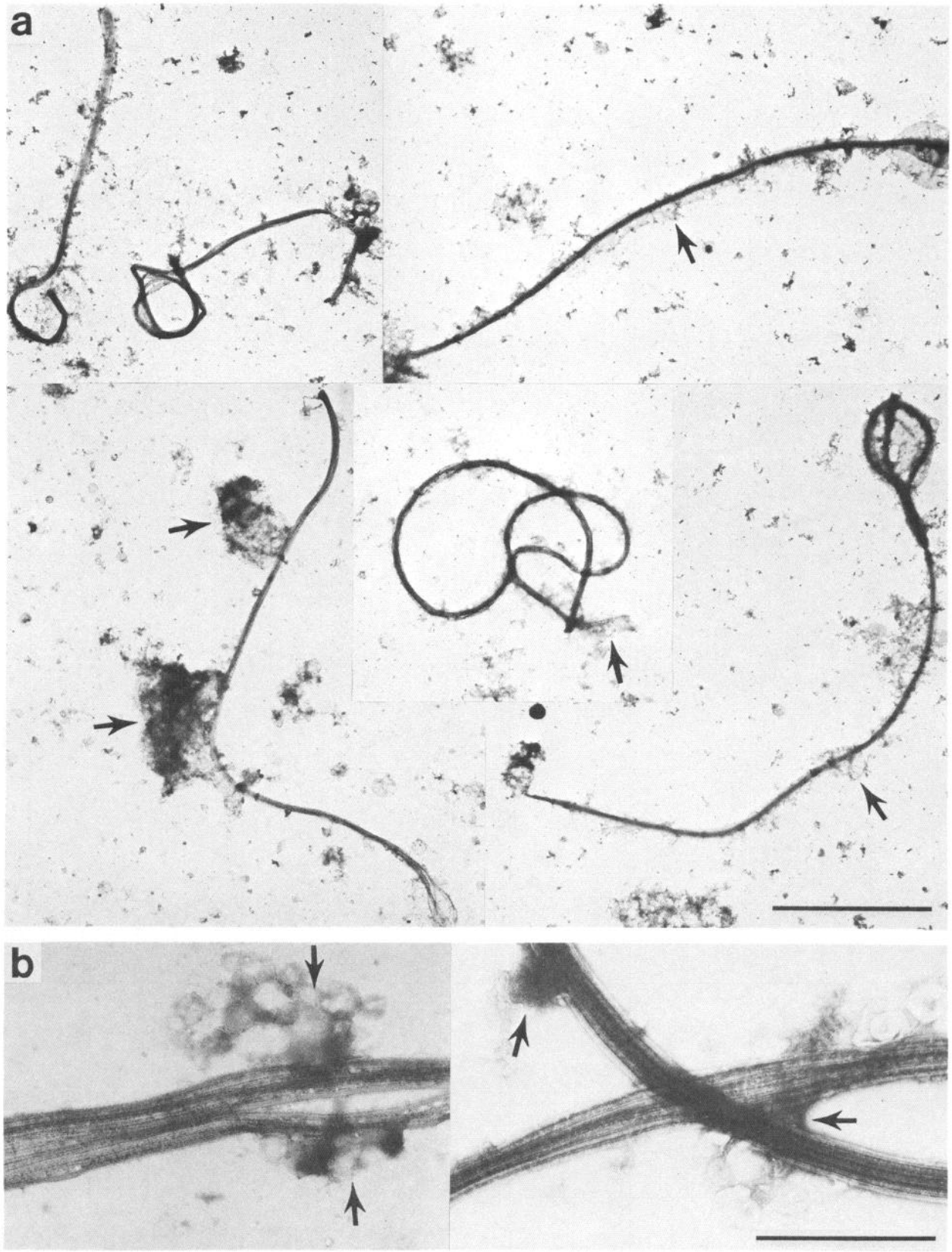

Figure 2. Electron micrographs of negatively stained cilia from the olfactory epithelium of *Rana catesbeiana*. *a,* Montage of 5 representative fields showing isolated olfactory cilia. In most places the ciliary plasma membrane appears to detach from the axonemes as small membrane fragments or vesicles (*arrows*), giving rise to many of the vesicular structures seen in Figure 1. *b,* Montage of 2 areas at higher magnification illustrating the ultrastructure of the cilia. Arrays of laterally aggregated microtubules can be clearly discerned. In a few places, indications of transverse connections between adjacent microtubules, presumably dynein arms, can be perceived. *Arrows* point to ciliary plasma membrane fragments associated with the axonemes. Bars, 5 μm (*a*); 1 μm (*b*).

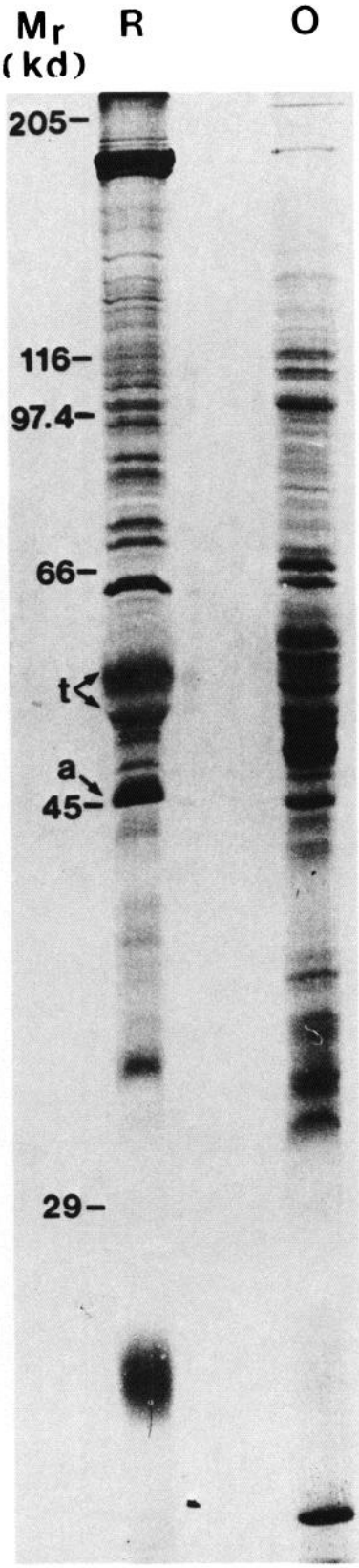

Figure 3. PAGE of isolated olfactory and respiratory cilia. *R*, Respiratory cilia; *O*, olfactory cilia. Each lane contains 30 μg protein. *t*, Tubulin dimer; *a*, actin.

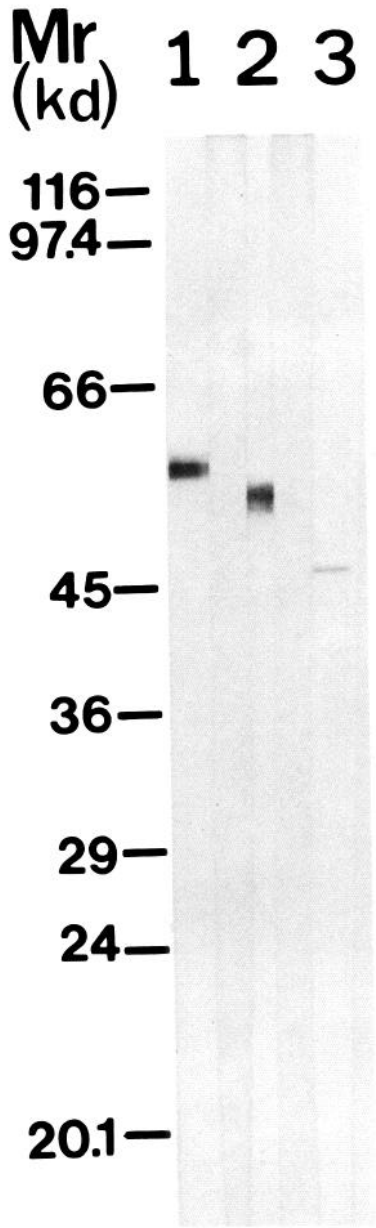

Figure 4. Identification of cytoskeletal proteins of olfactory cilia transferred onto a nitrocellulose membrane. Each strip contains approximately 10 μg of ciliary protein. *1*, Identification of α tubulin by monoclonal anti-α tubulin. *2*, Identification of β tubulin by monoclonal anti-β tubulin. *3*, Identification of actin by monoclonal anti-actin.

used previously by Chen and Lancet (1984) for the isolation of olfactory cilia.

Biochemical characterization of isolated olfactory cilia

PAGE in SDS of the olfactory cilia preparation shows a reproducible pattern of bands (Fig. 3). Respiratory cilia isolated by the identical procedure are shown on the same gel for comparison. The axonemal proteins, α and β tubulin, are particularly prominent in the band pattern of the respiratory cilia. In addition, actin is readily detectable, migrating with an apparent molecular weight of 46 kDa. A densely stained band at 185 kDa, possibly a spectrin-like cytoskeletal protein, is also prominent. As a percentage of the total ciliary protein, these cytoskeletal components are less conspicuous in the olfactory cilia. However,

they can be readily identified by immunoblotting, which reveals α and β tubulin, as well as actin, in the olfactory cilia as 55, 53, and 46 kDa bands, respectively (Fig. 4). At least 30 bands are readily distinguished on silver-stained gels of olfactory cilia, with particularly prominent groups of bands between 31 and 38 kDa and 48 and 54 kDa, in addition to major components at 64, 98, 105, and 110 kDa (Fig. 3).

To detect glycoproteins in isolated olfactory and respiratory cilia, we transferred the gel pattern onto a nitrocellulose membrane and probed it with concanavalin A. About 15 glycoprotein bands are discernible, mostly with apparent molecular weights greater than 55 kDa (Fig. 5). Staining of 3 faint bands, at 74, 78, and 120 kDa, cannot be prevented by the presence of 0.5 M α-methyl-D-mannoside; thus, their glycoprotein nature is ambiguous. Especially prominent staining is observed in a region of 56–65 kDa and of bands at 95 and 116 kDa. In contrast, respiratory cilia display only some faint glycoprotein bands, lacking the prominent bands observed in olfactory cilia (Fig. 5).

Previous reports have suggested the involvement of a GTP-dependent adenylate cyclase system in olfactory reception (Kurihara and Koyama, 1972; Menevse et al., 1977; Pace et al., 1985). We attempted to identify components of transductory GTP-coupling proteins (G-proteins) in isolated olfactory cilia by immunoblotting (for reviews, see Gilman, 1984, and Schramm and Selinger, 1984). Figure 6*A* shows a blot of olfactory cilia probed with rabbit antisera raised against a 39 kDa α subunit and a 36 kDa β subunit of G_o, a G-protein purified from bovine cortex (Huff et al., 1985). The antiserum against the α subunit of this G protein reveals a faint band with a molecular weight of 40 kDa. The antiserum against the β subunit stains a band with a molecular weight of 36 kDa. The presence of GTP-binding proteins is not detectable in respiratory cilia using these antisera under identical conditions (Fig. 6*B*).

If olfactory cilia initiate excitation of the receptor cell, they presumably undergo rapid dissipation of their transmembrane ion gradients during depolarization events. Accordingly, one might anticipate the presence of a Na^+/K^+-ATPase on the ciliary membrane necessary to maintain and restore the resting potential. This enzyme can, indeed, be identified in the isolated olfactory cilia preparation by immunoblotting with an antiserum raised against a Na^+/K^+-ATPase purified from chick kidney (Fig. 6*A*). The antiserum stains a diffuse region between 56 and 65 kDa, which overlaps the 56–65 kDa region identified with concanavalin A (see above) and corresponds to the expected migration pattern of the β subunit of the Na^+/K^+-ATPase. The α subunit expected to migrate as a 95–100 kDa species is not recognized by this antiserum. The antiserum reacts only slightly with respiratory cilia (Fig. 6*B*).

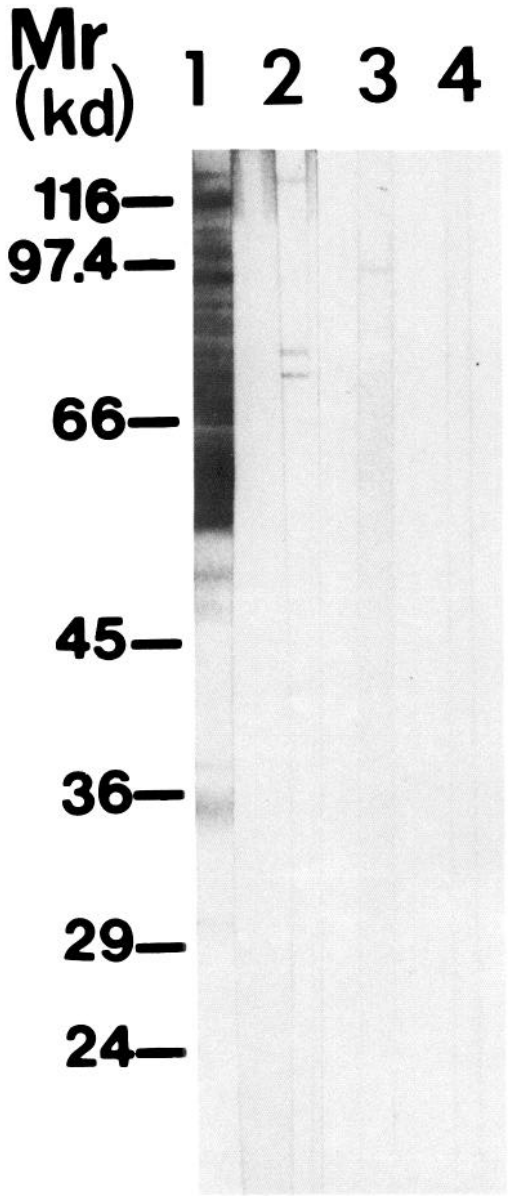

Figure 5. Identification of glycoproteins of olfactory and respiratory cilia transferred onto a nitrocellulose membrane. Each strip contains approximately 10 μg of ciliary protein. The strips were probed with concanavalin A (100 μg/ml; Sigma) in the absence (*1* and *3*) or presence (*2* and *4*) of 0.5 M α-methyl D-mannoside. Bound concanavalin A was visualized via an avidin-biotinylated HRP reaction using the Vectastain kit, as described in Materials and Methods, with deletion of the secondary antibody step. Lanes *1* and *2*, Olfactory cilia; lanes *3* and *4*, respiratory cilia.

Discussion

We have modified previously reported procedures to isolate olfactory cilia at high yield. Earlier protocols employed a 10% ethanol solution or other organic solvents during the calcium shock-induced detachment of the cilia (Chen and Lancet, 1984; Linck, 1973; Rhein and Cagan, 1980). We observed that the calcium shock alone, in the absence of organic solvent, is equally effective for detaching the cilia from the epithelium. Furthermore, we found the bullfrog, *Rana catesbeiana,* to be a more convenient source for the isolation of olfactory cilia than the smaller frogs *Rana pipiens* or *Rana ridibunda,* since this animal yields ~7× more ciliary protein per frog. During the isolation procedure most of the cilia undergo lysis, giving rise to isolated axonemal structures and reannealed plasma membrane vesicles (Figs. 1 and 2). The measured internal volume of these vesicles is substantial and would, in principle, allow measurement of radioisotope fluxes across these membranes.

It should be noted that the olfactory cilia preparation is somewhat contaminated by membrane fragments derived from other cellular organelles. We have used the mitochondrial marker, cytochrome oxidase, as an index for the extent of this contamination. In contrast to isolated retinal rod outer segments, where rhodopsin can be easily quantitated as a specific marker, it is difficult to precisely evaluate the purity of the olfactory cilia preparation, since no distinct biochemical markers specific for the ciliary membrane have hitherto been identified. The appearance of the preparation after negative staining suggests that a significant number of the observed membrane fragments are derived from the ciliary membrane (Fig. 2). Although we cannot fully exclude the possibility that the membrane proteins and glycoproteins that we identified in the olfactory cilia preparation are localized in part to contaminating organelles, this possibility

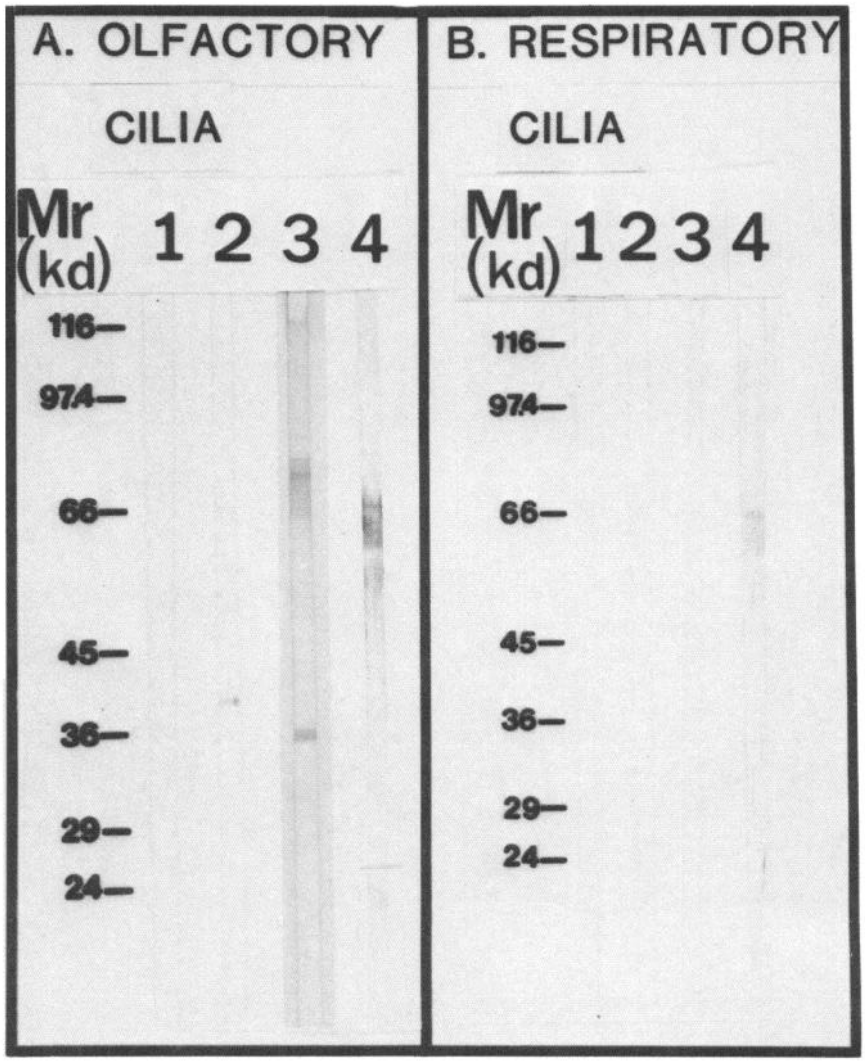

Figure 6. Identification of membrane proteins of olfactory and respiratory cilia transferred onto a nitrocellulose membrane. Each strip contains approximately 10 μg of ciliary protein. *1,* Strips incubated with a 200-fold dilution of normal rabbit serum. *2,* Strips incubated with a 200-fold dilution of antiserum against the α subunit of G_o from bovine cortex. *3,* Strips incubated with a 200-fold dilution of antiserum against the β subunit of G_o from bovine cortex. *4,* Strips incubated with a 500-fold dilution of antiserum against the Na^+/K^+-ATPase from chick kidney.

seems unlikely in view of the fact that these components are virtually undetectable in cilia derived from respiratory epithelium via the identical procedure.

Characteristics of the isolated olfactory cilia preparation are summarized in Table 1. In contrast to respiratory cilia, olfactory cilia possess a large number of glycoproteins. Chen and Lancet (1984) detected 4 major glycoproteins in cilia from *Rana ridibunda* at molecular weights of 55, 58, 95, and 120 kDa. These proteins resemble the prominent glycopeptides of 56–65, 95, and 116 kDa observed in our preparations.

A role for cAMP as second messenger in olfactory reception has been suggested by the observations that phosphodiesterase inhibitors reduce the amplitude of the electro-olfactogram (Menevse et al., 1977), that olfactory tissue contains high basal levels

Table 1. Characteristics of isolated cilia from the olfactory and respiratory epithelia of *Rana catesbeiana*

Characteristic	Yield (μg protein/frog ± SD)	Internal volume (μl/mg protein ± SD) (*n*)	Protein components: Glycoproteins	Cytoskeletal proteins: α Tubulin	β Tubulin	Actin	Membrane proteins: $G_o\alpha$	$G_o\beta$	Na^+/K^+-ATPase
Olfactory cilia	226 ± 19 (14)[a]	2.3 ± 0.5 (4)	Prominent with major bands at 56–65, 95, and 116 kDa	+	+	+	+	+	Prominent
Respiratory cilia	97 ± 15 (4)	N.D.[b]	Only faint bands detectable	+	+	+	–	–	Barely detectable

[a] The number of experiments performed is listed in parentheses. The yield of olfactory cilia from *Rana pipiens,* using the same isolation procedure, is 32 ± 10 μg/frog (average of 6 experiments using 89 frogs).

[b] N.D. = not determined.

of adenylate cyclase activity (Kurihara and Koyama, 1972), and that this activity may be stimulated by at least some odorants in a GTP-dependent manner (Pace et al., 1985; P. Sklar and R. H. Anholt, unpublished observations). Pace et al. (1985) have identified G-proteins in olfactory cilia using cholera toxin- and pertussis toxin-catalyzed ADP-ribosylation. These studies suggested the presence of a G-protein that mediates stimulation of adenylate cyclase, G_s, and, to a lesser extent, a G-protein that mediates inhibition of adenylate cyclase, G_i. The latter was resolved as a doublet band after ADP-ribosylation by pertussis toxin, known to use both G_i and G_o as substrates. We have used an antiserum raised against the α subunit of G_o from bovine cortex, which detects a single polypeptide species at an apparent molecular weight of 40 kDa. In addition, an antiserum raised against the $\beta\gamma$ complex of this G-protein reveals a single polypeptide band at 36 kDa (Fig. 6). In most systems studied, it is well established that the G_s and G_i species play a role in stimulation and inhibition, respectively, of adenylate cyclase (for reviews, see Gilman, 1984, and Schramm and Selinger, 1984). The role of G_o is less clear, and it has been suggested that this protein may regulate other transduction systems, such as the phosphatidylinositol cycle (Cockcroft and Gomperts, 1985; Huff et al., 1985). The exact nature and function of the G-proteins found in olfactory cilia and their degree of similarity to retinal transducin or hormonal GTP-coupling proteins remain to be investigated further. In addition to G-proteins, olfactory cilia appear to contain a Na^+/K^+-pump for the maintenance of ionic homeostasis.

The isolated olfactory cilia preparation can be considered analogous to the isolated rod outer segments used for the study of transduction mechanisms in the retina (for a review, see Stryer, 1983). Biochemical studies on isolated olfactory cilia may lead to the establishment of quantitative assays for olfactory recognition and signal transduction *in vitro* and enable a systematic dissection of the sequence of molecular events that take place between the arrival of the odorant at the cell surface and the generation of an action potential.

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