Cell cultures of neuroblasts from rat olfactory epithelium that show odorant responses

(olfaction/neurogenesis/cAMP/neuron-specific enolase/carnosine)

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Communicated by Gordon H. Sato, November 28, 1988

ABSTRACT We have developed procedures that permit isolation and propagation of clonal cell cultures from the olfactory epithelium of the 5- to 7-day-old rat that appear to represent the neuroblasts that repopulate the sensory neurons in the olfactory epithelium throughout life. The cell lines we report here synthesize neuron-specific enolase, which is a neuron marker, 43-kDa growth-associated protein, a protein associated with neuronal growth cones, and carnosine, a possible olfactory neurotransmitter. In two of the cell lines we have found dose-dependent cAMP accumulation following exposure to submicromolar concentrations of chemical odorants in the medium. These two cell lines show different patterns of odorant specificity when tested against a panel of six chemicals commonly used as test odorants. We anticipate that these and similarly derived cell lines will prove valuable in studying aspects of neurogenesis and olfaction.

The vertebrate olfactory epithelium (OE) contains primary sensory neurons that synapse in the olfactory bulbs and are responsible for transmitting chemosensory information to the brain. In the OE these cells are surrounded by sustentacular cells that are secretory and may also serve a glia-like function for the sensory neurons. At the base of this pseudostratified epithelium lie the basal cells, which divide and give rise throughout life to new sensory neurons (1, 2) and possibly also to new sustentacular cells (1–3).

Evidence has been accumulating that the transduction of odorant stimulation to neural signaling employs a second messenger mechanism that includes cAMP (4–7). The proteins involved in signal transduction are enriched in the cilia (4) that arise from the dendritic processes of the sensory neurons. Other mechanisms may be used. Apparently some odorants can gate cation channels directly in membranes prepared from the OE (8). But as yet there has been no direct biochemical demonstration of an odorant receptor or system of receptor molecules sufficiently diverse to account for the very high specificity exhibited in olfaction. Extracellular recordings made from frog OE show great diversity from cell to cell in the responses elicited from a sequence of odorants presented one at a time (9). Biochemical studies of cilia preparations, like studies of the whole OE, cannot resolve the contributions of individual cells; however, cloned cell cultures could help to define the biochemical differences among the various sensory cells.

The demonstration (1, 2) that the olfactory sensory neurons were renewed throughout life from a population of dividing stem cells has suggested that it might be possible to culture the stem cells or some neuron precursor. Past attempts to grow these cells in culture (3, 10, 11) did not yield strains that were differentiated sufficiently to suggest their use in the study of olfaction in vitro. We report here that two of three cloned cell strains, although they appear to be incompletely differentiated neurons in culture, have nevertheless responded sensitively and selectively to test odorants by accumulating cAMP. They also synthesize neuron-specific enolase (NSE; EC 4.2.1.11) (12), 43-kDa growth-associated protein (GAP-43), found in growth cones of developing neurons (13), and the characteristic dipeptide, carnosine, which may serve as a neurotransmitter (14). These cells have been in continuous culture for 2.5 years and may now be considered to be cell lines.

MATERIALS AND METHODS

Culture Medium. The basic culture medium has been Coon’s modified F-12 medium (15) further modified to contain no added calcium, 0.48 mM magnesium, and KCl reduced to 230 mg/liter. Fetal calf serum (GIBCO) was added to 6%. Bovine hypothalamus and bovine pituitary extracts were added to final concentrations of 150 and 50 µg of protein per ml, respectively. The tissue extracts were made by combining frozen tissue (Pel Freez Biologicals) and 200 mM Hepes buffer (pH 7.2) to yield 33% wt/vol in a Waring blender. (After 30 min of refrigeration the brei was remixed by a brief burst on the blender.) After centrifugation at 6000 × g for 1 hr, the supernatant fluid was clarified at 40,000 × g for 1 hr. The supernatant fluid (avoiding the translucent, viscous material just above the pellet) was frozen in liquid nitrogen and stored in the vapor phase. Other standard cell culture additives were included: Na-insulin (Elanco, Indianapolis), 1 µg/ml; human transferrin, 5 µg/ml; hydrocortisone, 0.01 µM; retinoic acid, 5 ng/ml (all Sigma); and gentamicin, 50 µg/ml (GIBCO). The complete medium, designated 4506, was analyzed (Nichols Institute, San Juan Capistrano, CA) and contains <0.1 mM free calcium. Where indicated in step-down experiments, Dulbecco’s modified Eagle’s medium (DMEM) (GIBCO) with added nonessential amino acids and gentamicin (2.0 mM calcium) was used as a medium promoting differentiation.

Establishment of Cell Cultures. Fischer rats (CDF (Fischer-344)/Cr1BR, Charles River Breeding Laboratories) 5–7 days postpartum were used. The region of the epithelium most rich in the olfactory neurons (dorsal and posterior in the nasal cavity) was freed using watchmaker’s forceps. The sheets of

Abbreviations: OE, olfactory epithelium; NSE, neuron-specific enolase; GAP-43, 43-kDa growth-associated protein; OMP, olfactory marker protein; RBM, reconstituted basement membrane; SNIF, sensory neuron in vitro fascimile.

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tissue were cut into pieces about 1 mm square and 0.5 mm thick. Three or four of these fragments were transferred to 35-mm tissue culture dishes and covered with 0.03 ml of reconstituted basement membrane (RBM) preparation (16) available as "matrigel" (Collaborative Research). This predominately type IV collagen solution gels overnight at 37°C and serves to hold the explanted OE against the surface of the culture dish and prevents its closure into an epithelial vesicle that would not attach. After culture under 4506 medium for 7–10 days at 36.5°C in a humidified incubator (6.5% CO2 atmosphere), several cell types had migrated from the explants and spread on the dishes and in the meshes of the gel. Primary cell cultures were established by digesting (37°C) the gel containing the outgrowths and remnant tissue fragments with 0.1% trypsin (1:300, ICN), 2% chicken serum (GIBCO), and collagenase (GIBCO) added to make 200 units/ml (15).

The cell cultures were diluted into 60-mm tissue culture dishes precoated 24 hr earlier with 0.03 ml of RBM using glass spacers. They were fed by exchange with fresh 4506 medium twice weekly. After two passages, single cell suspensions were plated for cloning at 10–106 cells per 60-mm plate. Cloning plates were fed with 4506 medium conditioned by incubating 12 ml for 24 hr in crowded plates of the "parental" cell populations. After 2–3 weeks, well-isolated, homogeneous colonies were selectively trypsinized and transferred to individual 60-mm dishes. Three of these cloned strains are described, named SNIF (sensory neuron in vitro fascimile), SNIF-12, -11, and -6. Each of these clones came from a different animal and was later recloned at least once.

Determination of Intracellular CAMP Levels After Exposure to Odorants. Cells were trypsinized, plated in RBM-coated 24-well cluster dishes (16 mm in diameter), and grown to near confluence. The cells were preincubated for 10 min at 37°C in 0.25 ml of assay medium: DMEM, without bicarbonate or glutamine, modified to contain 1 mM CaCl2 and 0.5 mM MgCl2 and supplemented with 20 mM HEPES (pH 7.4), 3 mg of heat-inactivated (56°C, 60 min) bovine serum albumin per ml, and 0.5 mM 3-isobutyl-1-methylxanthine (Aldrich). Equal volumes of assay medium containing odorants or other stimulants were added and incubated for 10 min longer. Medium was then removed, 1 ml of 0.1 M HCl containing 0.1 mM CaCl2 was added, and the plates were stored at −20°C. All determinations were performed in quadruplicate. Upon thawing, wells were neutralized by addition of 20 μl of 5 M NaOH, and cAMP was determined using a Rianen cAMP RIA kit (DuPont).

Stimulants. Odorants previously tested on purified cilia from OE (4, 5) were used: citral, 3,7-dimethyl-2,6-octadienitrile; coniferan, 2-tert-pentylcyclohexanol acetate; β-ionone, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one; and geranyl acetate, 3,7-dimethyl-2,6-octadiene-1-yl acetate (all from International Flavors and Fragrances, Union Beach, NJ); pyridine (J. T. Baker); camphor (Glyco, Williamsport, PA). Other cAMP stimulants were forskolin (Behring Diagnostics); cholera toxin (List Biological Laboratories, Campbell, CA); isoproterenol and prostaglandin E1 (Sigma).

Immunoblot Analysis. Cell cultures or rat tissues were homogenized in 10 mM sodium phosphate buffer (pH 7.5), 100 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% NaDdSO4, 50 μg of phenylmethanesulfonyl fluoride per ml, 10 μg of L-1-tosylamido-2-phenylethyl chloromethyl ketone per ml, 1 μg of aprotinin per ml, and 1 μg of leupeptin per ml (Boehringer Mannheim) at 4°C and centrifuged for 10 min at 16,000 × g. Protein was determined by bicinchoninic acid assay (Pierce). The cell-free extracts were stored at −80°C. Gel electrophoresis was done under denaturing conditions on 10% polyacrylamide (17). Electrophoretic transfer from polyacrylamide gels to nitrocellulose was performed as described (18) at 100 V and 150 mA for 1 hr at 4°C. Filters were blocked with 5% (wt/vol) nonfat dry milk, exposed to primary antibody, diluted in TBS (20 mM Tris-HCl, pH 7.5/500 mM NaCl) containing 10 μg of bovine serum albumin per ml, each step for 30 min at room temperature. Following three washes in TBS plus 0.05% Tween-20 (Bio-Rad), filters were incubated in second antibody diluted in TBS plus bovine serum albumin. Second antibodies used were rabbit anti-goat IgG crosslinked to horseradish peroxidase diluted 1:1,000, biotinylated goat anti-rabbit diluted 1:1,000, and streptavidin crosslinked to horseradish peroxidase diluted 1:1,000 (all Kirkegaard and Perry Laboratories, Gaithersburg, MD). Following washing, filters were developed in 0.5 mg of 4-chloro-1-naphthol per ml (Bio-Rad) in TBS containing 17% methanol and 0.015% H2O2. For GAP-43, electrophoresis was done as described (19), and filters were blocked in TBS plus 10% normal rabbit serum, exposed to primary antibody diluted in TBS plus 1% normal rabbit serum, and developed using the Vector ABC peroxidase kit (Vector Laboratories).

RESULTS

Cell Cultures. In selecting clones an attempt was made to choose representatives of the several cell types that appeared

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**Fig. 1.** (A) SNIF-11 cells cultured for 3 days in protein-rich medium 4506. Most are flat, or bipolar, epitheloid cells with short processes. (B) SNIF-11 cells cultured for 3 days in protein-free DMEM. Most cells are rounded, refractile cells starting to extend neuron-like processes. These become longer (> 200 μm) and thinner after 7–14 days in culture. (Bars = 100 μm.)
in primary cultures. Most of the colonies contained predominantly bipolar cells. In spite of reclonings and subclonings the populations grown from these cell strains always showed a variety of morphologies (Fig. 1A). This result is not unexpected if our cultures represent the stem cells (basal cells) or a member of the series of maturing sensory neurons.

In attempts to enrich for bipolar cells with extended processes, we found the most effective method was to change the medium from protein- and growth factor-rich, growth medium 4506 to simple factor and protein-free DMEM, a step-down procedure (20). In DMEM, nearly confluent cultures survive for months while the population remains static. After 1 or 2 weeks as many as 70% of the cells become rounded and show increased extension of neuron-like processes (Fig. 1B) as neuroblastoma cells do when shifted to serum-free medium (20).

**Characterization of the Cell Strains.** Our approach has been based on (i) a search for specific markers for neuron and olfactory cells and (ii) functional assays for odorant-dependent second messenger response (cAMP accumulation).

**Immunological Markers.** Since Margolis (21) described olfactory marker protein (OMP), identification of mature olfactory neurons in OE and in culture has depended upon its demonstration. OMP is an 18.7-kDa cytosolic protein of unknown function that is abundant in the cell bodies and neurites of mature olfactory sensory neurons. It has been identified by immunofluorescence in organ cultures of OE from fetal rats (10) and reported at least transiently in mouse OE and cerebellum. It reacts with anti-OMP serum (unpublished) or a member (10, 11, 22). Upon immunoblot analysis, however, it was evident that the SNIF cell lines lacked an OMP band but did contain a strongly reacting protein migrating at about 40 kDa (Fig. 2). This 40-kDa protein also appeared in rat cell lines from other tissues. It was seen, at much lower levels, in extracts of rat OE and cerebellum. Because OMP antiserum raised in three other animals each stained different bands in addition to authentic OMP on similar blots (data not shown), we decided that these non-OMP specificities most likely were due to minor contaminations in the antigen preparations and not due to immunological relationships among these proteins and OMP.

NSE, a marker for neurons and neuroendocrine cells (12), is easily demonstrated in frozen sections of OE from 5- to 7-day-old rat pups. Immunostaining is confined to the mature sensory neurons and their proximate precursors but is not present in the sustentacular or basal cells. A gradient of fluorescence intensity is lowest near the basement membrane, becoming higher toward the lumen, and is highest in the mature sensory neurons. All of our cell lines contain NSE, as shown for two of them in Fig. 3A. Cultures grown in 4506 medium have lower specific activity than do cultures that have been shifted from protein-rich growth medium to DMEM. The unequivocal presence of this neuron marker in our cell lines and its immunohistological absence from other cell types in frozen sections of the OE strengthens our confidence that the SNIF cell lines are derived from neuronal precursors.

GAP-43 is localized in the membranes of the growth cones of developing neurons (13) and has been demonstrated in the olfactory tract (23). We have identified a protein with the same mobility that reacts with GAP-43 antiserum on immunoblots (Fig. 3B) prepared from extracts of SNIF-11 and -12. A protein with these properties, presumably GAP-43, may also appear in certain glial cell cultures (24) and in the C6 rat glioma (Fig. 3B); however, immunoblots of SNIF-11 and -12 do not show the glial marker, glial fibrillary acidic protein (data not shown). The presence of putative GAP-43 in the absence of the glial marker constitutes further evidence that the SNIF cell cultures contain maturing neurons.

**Carnosine Synthesis.** The dipeptide carnosine (β-alanyl histidine) has been identified in the olfactory nerve layer of the olfactory bulbs and OE and has been implicated as a possible neurotransmitter in the vertebrate olfactory system (14). We have demonstrated incorporation of 3H-labeled β-alanine into chromatographically purified carnosine from SNIF-11 cells that had been grown in DMEM for 2 weeks.

![Image](image-url)

**Fig. 2.** Immunoblot of tissue and cultured cell protein extracts (10 µg) stained with goat anti-rat OMP serum diluted 1:500. OMP is only in the OE, not in the cerebellum (CBL) or in any of the cultured cells. The slower migrating protein (40 kDa) that reacts strongly with anti-OMP serum appears in all tissues and cells shown. Paired lanes are from two different litters of 5-day-old rats for OE or two passages of SNIF cells. Control cells are C6 (rat glioma), FRTL-5 (rat thyroid epithelial cells), and BRL-30E (rat liver epithelial cells).

**Fig. 3.** (A) Immunoblot of tissue and cultured cell protein extracts (10 µg) stained with rabbit anti-rat NSE (Polyscience) diluted 1:1000. Cultures of SNIF-12 and -11 were grown in protein-rich 4506 medium (lanes a) or protein-free DMEM (lanes b) for 2 weeks. The NSE band is apparently darker in lanes b. The lane marked NSE contains 250 ng of purified rat NSE (Polyscience). Other tissues and cells are as in Fig. 2. (B) Immunoblot of tissue and cultured cell protein stained with sheep anti-rat GAP-43 serum (from L. I. Benowitz, ref. 13) diluted 1:1000. Cells were cultured as in A and homogenized (glass pestle) in a buffer similar to that described in the text but without detergents. Protein from the 100,000 × g pellet was resuspended in sample buffer (17), and 25 µg was applied to each lane. Tissue protein was similarly prepared. N-18 is C-1300 mouse neuroblastoma.
Table 1. Intracellular cAMP accumulation response of three SNIF cell lines (SNIF-12, SNIF-11, SNIF-6) to stimuli

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>Control</th>
<th>Isoproterenol</th>
<th>Prostaglandin E₁</th>
<th>Forskolin</th>
<th>Cholera toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP, pmol/mg of protein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exp. 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.3 ± 0.5</td>
<td>11.8 ± 1.3</td>
<td>10.3 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>56.7 ± 0.4</td>
<td>115 ± 20</td>
<td>18.3 ± 1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostaglandin</td>
<td>4,450 ± 356</td>
<td>8,450 ± 1,103</td>
<td>1,790 ± 200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forskolin</td>
<td>332 ± 50</td>
<td>380 ± 45</td>
<td>212 ± 18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholera toxin</td>
<td>2,930 ± 106</td>
<td>295 ± 13</td>
<td>1,041 ± 83</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When assaying in vitro for adenylly cyclase, sonicated membrane preparations from SNIF-12 were lower in specific activity by a factor of ~500 than those from isolated rat olfactory cilia. Values are expressed as mean ± SEM. In experiment 1, stimulants were 0.01 mM, except that cholera toxin was 1 μM.

(data not shown). Supernatant extracts of homogenized cells supported incorporation of [¹⁴C]histidine into carnosine at 105 pmol/mg of protein/hr, ~60% of what we found from OE of 5- to 7-day-old rats; these rates are similar to those reported for OE from adult mice (25).

Functional Assays. Biochemical studies (3–5) clearly have demonstrated an odorant-dependent increase of adenylly cyclase activity in preparations of cilia from frog or rat OE. It seemed that the SNIF cell strains, derived from the OE, might be capable of responding to odorant stimuli by production of a second messenger such as cAMP. Therefore, we measured the accumulation of cAMP in cells incubated briefly with various odorants. Table 1 shows cAMP responses of three SNIF cell lines to standard nonodorant stimulants of cAMP production; Table 2 compares the stimulants in the case of SNIF-12 cells to examples of odorant responses. As shown by the responses to cholera toxin and forskolin (Table 1), these cells, especially SNIF-12, are rich in guanine nucleotide-binding protein-linked adenylly cyclase. Just as in the assays for adenylly cyclase activity in cilia preparations, our cAMP accumulation responses to odorants, when present, are small relative to those of nonodorant stimulants. Both assays typically show odorant responses between 1.3 and 2 times the unstimulated control values (Tables 1 and 2; Fig. 4). The odorant responses of our cells fluctuate greatly from well to well, as shown by the large standard errors, requiring at least quadruplicate determinations at each assay point.

Fig. 4 shows the dependence of cAMP accumulation on the concentration of citralva in SNIF-12 cells. There is a peak of >3-fold stimulation of cAMP levels at 0.1 μM citralva. This concentration is 3–4 orders of magnitude more dilute than the odorant concentrations required for yielding highest stimulation of adenylly cyclase in membranes from isolated olfac-

Table 2. Intracellular cAMP accumulation response of the SNIF-12 cell line to stimuli

<table>
<thead>
<tr>
<th>Stimulant</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
<th>Exp. 4</th>
<th>Exp. 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37 ± 1.1</td>
<td>29 ± 2.2</td>
<td>50 ± 6.0</td>
<td>34 ± 5.8</td>
</tr>
<tr>
<td>Citralva</td>
<td>53 ± 9.5*</td>
<td>49 ± 3.6*</td>
<td>151 ± 32*</td>
<td>111 ± 8.5*</td>
</tr>
<tr>
<td>Coniferan</td>
<td>44 ± 3.2*</td>
<td>49 ± 3.6*</td>
<td>151 ± 32*</td>
<td>111 ± 8.5*</td>
</tr>
<tr>
<td>Camphor</td>
<td>38 ± 3.6</td>
<td>49 ± 6.8*</td>
<td>219 ± 8.6*</td>
<td></td>
</tr>
<tr>
<td>Pyridine</td>
<td>49 ± 6.8*</td>
<td>219 ± 8.6*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

See legend to Table 1. Values are expressed as mean ± SEM. In experiment 2, all odorants were 1 μM and isoproterenol was 0.01 mM. In experiments 3–5, citral was 0.1 μM. Values are based on t statistics after logarithmic transformation. In experiment 2, analysis of variance with adjustment for multiple comparison (26) yields: citralva, \( P < 0.01 \); pyridine, \( P < 0.05 \); coniferan, \( P > 0.05 \).

* \( P < 0.002 \).

\( P > 0.05 \).

\( P < 0.02 \).

\( P < 0.005 \).

\( P > 0.05 \).

\( P > 0.02 \).

\( P < 0.002 \).

\( P < 0.005 \).

\( P > 0.05 \).

\( P > 0.02 \).

Table 3. Summary of cAMP tests

<table>
<thead>
<tr>
<th>Odorant</th>
<th>SNIF-12</th>
<th>SNIF-11</th>
<th>SNIF-6</th>
<th>N-18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citralva</td>
<td>7/15</td>
<td>0/7</td>
<td>0/4</td>
<td>0/6</td>
</tr>
<tr>
<td>β-Ionone</td>
<td>0/4</td>
<td>2/3</td>
<td>0/3</td>
<td>0/4</td>
</tr>
<tr>
<td>Geranyl acetate</td>
<td>0/4</td>
<td>0/3</td>
<td>0/4</td>
<td>0/2</td>
</tr>
<tr>
<td>Coniferan</td>
<td>1/2</td>
<td>0/2</td>
<td>0/3</td>
<td>0/2</td>
</tr>
<tr>
<td>Pyridine</td>
<td>1/1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Camphor</td>
<td>0/3</td>
<td>0/2</td>
<td>0/2</td>
<td></td>
</tr>
</tbody>
</table>

The numerator indicates an independent experiment showing a significant \( P < 0.05 \) odorant-stimulated cAMP accumulation after 10 min of incubation with the indicated odorant. The denominator indicates the total number of experiments. The SNIF-12 cell line is significantly different from the others \( \left(P < 0.001\right) \); however, none of the other cell lines is distinguishable. Negative responses measured when that passage of cells did respond to another odorant are marked with a minus sign. A question mark indicates that all measurements done on cells of that passage showed no odorant responses and are therefore inconclusive.

DISCUSSION AND CONCLUSIONS

These results show in at least one line, SNIF-12, and probably in a second, SNIF-11, that an odorant-dependent cAMP response has occurred selectively and in an apparently regulated fashion at levels more sensitive than previously reported in isolated cilia membrane. The biphasic dose-response curve, seen in Fig. 4, which we have observed twice in SNIF-12, suggests that SNIF cells, unlike cilia membranes, can regulate levels of cAMP not only by an odorant-dependent increase in cAMP production at low odorant concentrations but also by an odorant-dependent inhibition of cAMP production at high concentrations. This may be similar to the desensitization described for other receptor-mediated adenylly cyclase systems such as catecholamines and glucagon (27).
The cAMP accumulation responses of SNIF-12 and -11 are sensitive in that they are maximal at submicromolar levels of odorant. Odorant concentrations in the 0.05–1.0 mM range must be used with ciliary membrane preparations (4–6) and other odorant-responsive model cell systems (28, 29). It is difficult to compare the adenyl cyclase determinations made on cilia membranes with our cAMP accumulation data for whole cultured cells. Furthermore, the pool of cilia undoubtedly represents an average of all cellular specificities in the OE, and it may be that only a minority of the cilia responds efficiently to a given odorant. We might expect that a cloned cell population, such as SNIF-12, would be able to respond to a given odorant with a higher level of cAMP than that of the average of a mixed population. We find maximal responses to a concentration of odorant that is lower by a factor of 1000, but the total amount of cAMP accumulated is still very small (about 1/10th of that produced by forskolin). It may turn out that odorant responses are conducted at smaller intracellular cAMP levels than are evoked by hormones or that SNIF cells are not uniformly differentiated under our culture conditions. They are incompletely differentiated (cilia are not seen, OME is not present). From morphology we believe that there must be variable numbers of partially differentiated cells in each culture, which could explain the high variability and erratic responses we have encountered.

Likewise, odorant discrimination, at least in a binary sense, is not expected from the pooled population response of a ciliary preparation. We consider it important that two of the SNIF cell lines respond to different odorants in what appears to be an all-or-none fashion (within the resolution provided by our rather noisy assay), whereas none of the cell strains responds to all odorants. With the exception of pyridine the odorants we have used gave adenyl cyclase responses in ciliary membrane preparations (5).

SNIF-12 and -11 are stem cell populations. They are very different from the usual differentiated cell systems: they consist at all times of a mixture of cells in various stages of replication and differentiation. We do not know yet how to prepare populations of SNIF-11 or -12 so that they will respond maximally to odorant stimulation. Just under half of our assays with SNIF-12 and citralva have shown significant (P < 0.05) cAMP responses (above basal levels). Those responses, when they occur, may be quite vigorous (Fig. 4) or weaker (Table 2). The other assays for NSE, GAP-43, and carnosine synthesis can be induced routinely to easily detectable levels by simple shift-down to plain DMEM; this strategem has not yet made the odorant response data less erratic.

Two non-olfactory cell lines have been shown to respond to test odorants. The C-1300 neuroblastoma, strain N-18, was proposed as a model cell system for taste and olfaction (29) because these cells depolarize when exposed to millimolar concentrations of several test odorants. We have included N-18 as a control in some of our experiments but have found no cAMP accumulation responses. Lerner et al. (28) have found cAMP accumulation and accompanying dispersion of the melanosomes in cultured frog melanocytes as responses elicited by a variety of test odorants. Again, maximal responses were obtained in the 0.05–1.0 mM range. An even larger disparity between the threshold sensitivities of human subjects compared to the melanocyte cultures led Lerner et al. (28) to suggest that separate physiological responses may be involved. Perhaps, when these phenomena can be studied more completely in cloned cell strains, it will turn out that the millimolar responses are less discriminating and less likely to involve the reactions that confer specificity in olfaction than are the submicromolar responses.

SNIF cells have some neural properties and some olfactory properties and act like neuroblasts. The availability of cloned olfactory cell lines with odorant responses should greatly assist in identifying the molecules that confer specificity on the olfactory responses. We believe that these and similarly derived cell lines can provide valuable insights into neurogenesis in general and olfaction in particular.

We gratefully acknowledge the help of Dr. Frank L. Margolis and Larry I. Benowitz, who have generously shared their antisera and expert advice. We thank Dr. Robert E. Tarone for help with statistics and Drs. Chandler M. Fulton and Werner A. Klee for critically reading the manuscript and for helpful discussions. We thank Ms. Lori Redmond and Ms. Susan A. Fedak for their excellent technical assistance. We are grateful to Drs. Hynda K. Kleinman and George R. Martin for providing RBM and to International Flavors and Fragrances for their gift of purified odorants.