Human Services, National Institutes of Health, Bethesda (1980).
21. R. A. Hegele *et al.*, *N. Engl. J. Med.* **315**, 1509 (1986).
22. L.-S. Huang and J. L. Breslow, *J. Biol. Chem.* **262**, 8952 (1987).
23. R. J. Havel, H. A. Eder, J. H. Bradgon, *J. Clin. Invest.* **34**, 1345 (1955).
24. D. W. Bilheimer, S. Eisenberg, R. I. Levy, *Biochim. Biophys. Acta* **260**, 212 (1972).
25. G. Scatchard, *Ann. N.Y. Acad. Sci.* **51**, 660 (1949).
26. P. J. Munson and D. Rodbard, *Anal. Biochem.* **107**, 220 (1980).
27. We thank L. K. Curtiss for the MB-19 antibody, W. E. Nance for helpful criticisms, and L. Duda for preparing the manuscript. Supported in part by NIH grants (HL33714, HL32435, HL36461, and CA29502), a Clinical Investigator Award (HL02034), a Fogarty International Research Fellowship (TWO3960), a General Clinical Research Center grant (RR00102), and general support from the Pew Trusts. J.L.B. is an Established Investigator of the American Heart Association.

29 July 1988; accepted 23 December 1988

Identification of a Thyroid Hormone Receptor That Is Pituitary-Specific

RICHARD A. HODIN,*† MITCHELL A. LAZAR,* BRUCE I. WINTMAN, DOUGLAS S. DARLING, RONALD J. KOENIG,‡ P. REED LARSEN, DAVID D. MOORE, WILLIAM W. CHIN

Three cellular homologs of the v-*erbA* oncogene were previously identified in the rat; two of them encode high affinity receptors for the thyroid hormone triiodothyronine (T_3). A rat complementary DNA clone encoding a T_3 receptor form of the ErbA protein, called r-ErbAβ-2, was isolated. The r-ErbAβ-2 protein differs at its amino terminus from the previously described rat protein encoded by c-*erbA*β and referred to as r-ErbAβ-1. Unlike the other members of the c-*erbA* proto-oncogene family, which have a wide tissue distribution, r-*erbA*β-2 appears to be expressed only in the anterior pituitary gland. In addition, thyroid hormone downregulates r-*erbA*β-2 messenger RNA but not r-*erbA*β-1 messenger RNA in a pituitary tumor–derived cell line. The presence of a pituitary-specific form of the thyroid hormone receptor that may be selectively regulated by thyroid hormone could be important for the differential regulation of gene expression by T_3 in the pituitary gland.

THE NUMEROUS EFFECTS OF THYROID hormone on vertebrate tissues are thought to be mediated through binding to a nuclear receptor protein (*1*). The protein products of the c-*erbA* proto-oncogene have been identified as nuclear thyroid hormone receptors (*2, 3*). In the rat, three c-*erbA*–related cDNAs have been described and their encoded proteins divided into α and β forms on the basis of their predicted amino acid sequences (*4–8*). The r-*erbA*α-1 and r-*erbA*α-2 mRNAs represent alternative splice products of a single r-*erbA*α gene, which encodes proteins that are identical for the first 370 amino acids and then diverge completely (*5–7*). This divergent region spans the putative ligand-binding domain, probably accounting for the fact that the protein encoded by r-*erbA*α-1 binds T_3, whereas the protein encoded by r-*erbA*α-2 does not bind T_3. Like r-ErbAα-1, r-ErbAβ-1 binds T_3 with high affinity (*8*). The c-*erbA*–related mRNAs are expressed with differing abundance in virtually all rat tissues. The r-*erbA*α-1 mRNA (5 kb in size) is most abundant in skeletal muscle and brown fat, whereas the r-*erbA*α-2 mRNA (2.6 kb) is most highly expressed in brain and hypothalamus (*5*). The r-*erbA*β-1 mRNA (6.2 kb) is highly expressed in kidney and liver (*8*). We now report the isolation of a cDNA encoding a novel thyroid hormone receptor, r-ErbAβ-2, whose mRNA is detected only in the anterior pituitary gland. In addition, we find that the two r-*erbA*β mRNAs are differentially regulated by T_3 in GH_3 cells, a pituitary tumor–derived cell line.

The nucleotide and deduced amino acid sequences of the r-*erbA*β-2 cDNA are shown in Fig. 1A. The protein is schematized in Fig. 1B and compared to the other three rat c-ErbAs. The r-ErbAβ-2 protein has no similarity to r-ErbAβ-1 or either of the rErbA-α proteins at its amino terminus, but is identical to r-ErbAβ-1 from amino acid 147 to the carboxyl end, including the putative DNA- and ligand-binding regions. The identity between r-ErbAβ-1 and r-ErbAβ-2 begins one amino acid on the amino-terminal side of the point where the similarity between r-ErbAβ-1 and the r-ErbAα species begins—13 amino acids on the amino-terminal side of the first cysteine residue in the putative DNA-binding region.

The r-*erbA*β-2 cDNA insert was subcloned into the vector Bluescript and transcribed into RNA, which was translated in reticulocyte lysates. A protein of approximately 62 kD was produced, in agreement with that predicted from the nucleotide sequence (Fig. 2A). The r-ErbAβ-2 protein bound T_3 with an affinity constant (K_d) of 1.1 ± 0.4 n*M* (mean ± SEM) (Fig. 2B), which is similar to that of the endogenous T_3 receptor (*9*). In addition, competition experiments with thyroid hormone analogs showed that the affinity of the r-ErbAβ-2 protein was greatest for 3,5,3′-triiodothyrocetic acid (triac), followed by T_3, T_4, and rT_3 (*10*), the same as that of the r-ErbAβ-1 protein (*8*). These data are consistent with r-*erbA*β-2 encoding a functional T_3 receptor.

We used a transient transfection system to evaluate further the ability of r-ErbAβ-2 to function as a T_3 receptor (*11*). In the choriocarcinoma JEG-3 cell line, expression of a CAT reporter plasmid containing a rat growth hormone thyroid hormone response element was unchanged by T_3 treatment in the absence of a cotransfected *erbA*-containing plasmid. However, when cotransfected with 2 μg of an r-*erbA*β-2–containing plasmid (pCDMerbAβ-2), CAT expression was increased by a factor of 7.1 ± 2.1 (mean ± SEM) by treatment with 10 n*M* T_3. Identical experiments with an r-*erbA*β-1–containing plasmid (pCDMerb62) resulted in similar levels of T_3 induction; CAT expression was increased by a factor of 7.3 ± 1.1. When the vector plasmid alone (PCDM8) was cotransfected, no change in CAT expression was caused by T_3 treatment. Thus, as has been shown for r-ErbAβ-1 (*8*) and r-ErbAα-1 (*6*), r-ErbAβ-2 appears to represent a functional T_3 receptor.

Hybridization analyses of RNA from various rat tissues were performed with probes specific for the r-*erbA*β-1 or r-*erbA*β-2 mRNAs as well as a probe derived from the sequences common to the two cDNAs (r-*erbA*β-common). The r-*erbA*β-1 mRNA is 6.2 kb and is most abundant in brown fat and heart (Fig. 3). The relative intensities of the hybridization signals detected with the r-*erbA*β-common probe were similar to those

R. A. Hodin, M. A. Lazar, B. I. Wintman, D. S. Darling, W. W. Chin, Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Howard Hughes Medical Institute and Harvard Medical School, Boston, MA 02115.
R. J. Koenig and P. R. Larsen, Thyroid Division, Brigham and Women's Hospital, Howard Hughes Medical Institute and Harvard Medical School, Boston, MA 02115.
D. D. Moore, Department of Molecular Biology, Wellman 9, Massachusetts General Hospital, Boston, MA 02114.

*R. A. Hodin and M. A. Lazar contributed equally to this work and should both be considered first authors.
†To whom correspondence should be addressed.
‡Present address: University of Michigan Medical Center, Division of Endocrinology and Metabolism, 1500 East Medical Center Drive, 3920 Taubman Center, Ann Arbor, MI 48109–0354.

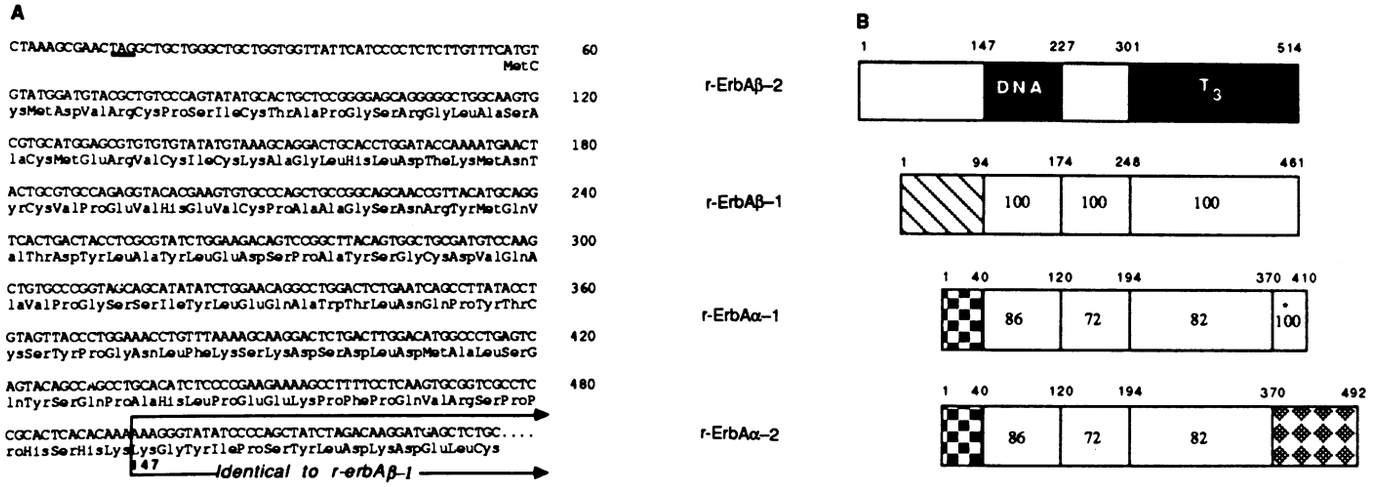

Fig. 1. (**A**) Nucleotide sequence of the r-*erbA*β-2 cDNA and deduced amino acid sequence. An in-frame stop codon is underlined. Amino acid designation begins at the first potential initiator methionine. Beginning at base pair 495, the r-*erbA*β-2 and r-*erbA*β-1 nucleotide sequences are identical throughout the full length of the cDNAs (the r-*erbA*β-2 cDNA extends seven nucleotides farther at the 3′ end). Two rat GH_3 cell cDNA libraries (*5, 8*) were initially screened with a fragment from the r-*erbA*α-2 cDNA clone and subsequently with a 5′ end fragment from a non–full-length r-*erbA*β-2 cDNA clone. Three positive clones corresponding to the r-*erbA*β-2 mRNA were obtained. The cDNA inserts were subcloned into the vector Bluescript (Stratagene) and sequenced by the dideoxy method of Sanger (*22*). Restriction fragments from two overlapping cDNAs were fused to construct a full-length r-*erbA*β-2 clone. The sequence depicted is that of the full-length cDNA. (**B**) Schematic representation of the r-*erbA*–derived amino acid sequences. The putative DNA and T_3 binding domains are designated by analogy to the glucocorticoid receptor. In each case, the numbers above the boxes refer to the amino acid sequence starting with the first in-frame methionine. Numbers within the boxes refer to percent nucleotide identity to r-*erbA*β-2. The r-ErbAα-1 protein contains three more amino acids at its carboxyl terminus than the r-ErbAβ proteins.

seen with the r-*erbA*β-1 probe in all tissues except the pituitary, which appeared to have less r-*erbA*β-1. The r-*erbA*β-2 probe also detected a 6.2-kb mRNA (Fig. 3C), and this may explain why the r-*erbA*β-common probe did not distinguish between the two r-*erbA*β species. The r-*erbA*β-2 mRNA was detected only in the anterior pituitary gland. Despite a 15 times longer exposure than that used for the autoradiogram in Fig. 3C, no hybridization with the r-*erbA*β-2 probe was detectable in other tissues. From these data we estimate that the r-*erbA*β-2 mRNA is at least 150 times more abundant in pituitary than in any other tissue examined. Experiments with r-*erbA*β-2 cDNA– and cRNA–derived probes yielded identical results. In addition, no r-*erbA*β-2 mRNA was detected in testis, kidney, skeletal muscle, or adrenal gland, although r-*erbA*β-1 mRNA was present (*12*). Therefore, unlike the other r-*erbA* gene products, which are expressed in virtually all tissues, the r-*erbA*β-2 mRNA appears to be limited to the anterior pituitary, as determined by Northern analyses.

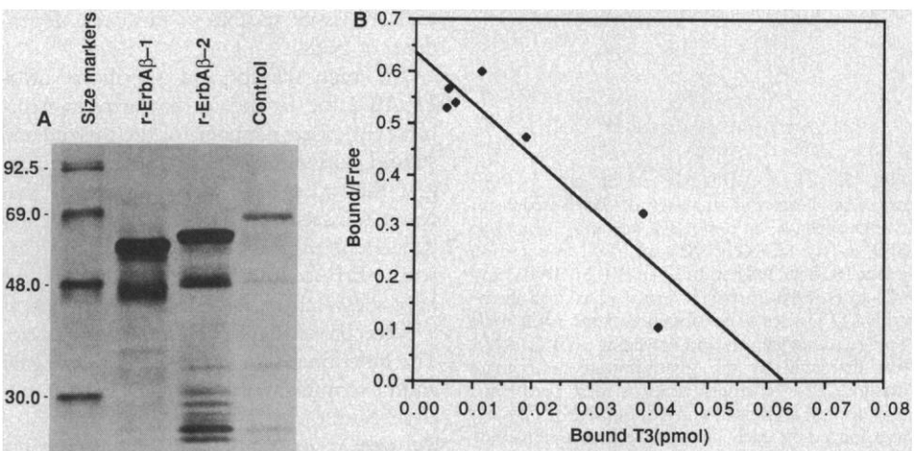

Fig. 2. (**A**) In vitro translation of the r-*erb-A*β clones. Fluorograph of an SDS-polyacrylamide gel after electrophoresis of [^{35}S]methionine-labeled r-Erb-Aβ-1 and r-Erb-Aβ-2 in vitro translation products. Two high molecular weight translation products were obtained with r-*erbA*β-2, the larger of which is approximately 62 kD, in agreement with that predicted from the nucleotide sequence. The control refers to unprogrammed reticulocyte lysates. Approximately 50,000 trichloroacetic acid–precipitable counts per minute were loaded in each lane. Molecular size markers are expressed in kilodaltons. The band at the bottom of each lane represents unincorporated [^{35}S]methionine moving with the ion front. (**B**) T_3 binding of r-ErbAβ-2 protein. Binding studies of [^{125}I]T_3 to r-ErbAβ-2 were subjected to Scatchard analysis to determine the affinity constant of the in vitro translated protein product (*23*). Labeled T_3 (0.1 n*M*) was incubated with unlabeled T_3 at 0 to 100-fold excess. Each point represents the mean of duplicate reactions. The binding reactions were carried out as described (*5*). The Scatchard plot depicts the results of a single experiment (K_d, 0.46 n*M*), representative of three separate binding studies.

In GH_3 cells, as in anterior pituitary, the r-*erbA*β-common probe hybridizes to an apparent single 6.2-kb species (*13*), which actually consists of both r-*erbA*β-1 and r-*erbA*β-2 mRNAs. We found earlier that r-*erbA*β mRNA levels (with the use of a common probe) in GH_3 cells decrease by approximately 50% after 24 hours of incubation with 10 n*M* T_3 (*13*). Using probes specific for either r-*erbA*β-1 or r-*erbA*β-2, we find a dramatic and specific effect of T_3 on r-*erbA*β-2 expression (Fig. 4A). In four separate experiments, the r-*erbA*β-2 mRNA levels decreased to a mean of 14% ± 3% (SEM) of control values ($P < 0.001$) after a 24-hour treatment with T_3. The downregulation of r-*erbA*β-2 mRNA was time dependent, in that an intermediate level of mRNA was detected after 6 hours of incubation with T_3 (decrease to 36% ± 7% of control). In contrast, r-*erbA*β-1 mRNA tended to increase slightly (up to twice control levels in some experiments) (Fig. 4B).

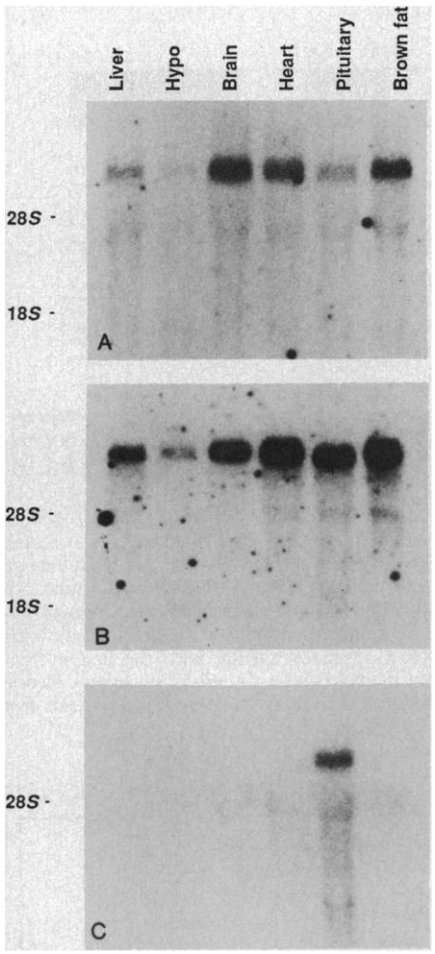

Fig. 3. Tissue distribution of the r-*erbA*β mRNAs. Northern analyses of RNA from rat tissues with (**A**) an r-*erbA*β-1–specific probe (1 to 550 in the cDNA), (**B**) an r-*erbA*β-common probe (515 to 2443 in the r-*erbA*β-2 cDNA), and (**C**) an r-*erbA*β-2–specific probe (1 to 515 in the cDNA). Tissues were obtained from adult male Sprague-Dawley rats and stored at −70°C. RNA was extracted by the guanidinium thiocyanate method (*24*). Northern analyses were performed by standard techniques (*25*). Total RNA (10 μg) was loaded in each lane and ethidium bromide staining confirmed equal loading of RNA per lane. The cDNA probes (r-*erbA*β-1–specific and r-*erbA*β-common) and cRNA probes (r-*erbA*β-2–specific) were labeled with ^{32}P, typically to a specific activity of 5×10^8 cpm per microgram of DNA. The autoradiograms in (A) and (B) represent 4-day exposures and the *erbA*β-2 autoradiogram represents a 12-hour exposure. Exposures of up to 8 days failed to reveal hybridization to the r-*erbA*β-2 mRNA from tissues other than the anterior pituitary. Hypo, hypothalamus.

The existence of r-ErbAβ-2 has a number of implications. The 1098-bp region of 100% identity between the r-*erbA*β-2 and r-*erbA*β-1 coding sequences, in addition to the 2898-bp identical region of 3′ untranslated sequences, suggests that the two mRNAs are derived from a single gene. Genomic DNA blotting analyses are consistent with this hypothesis (*14*). Thus, the tissue-specific expression and thyroid hormone regulation of r-*erbA*β transcripts may be due to differential activity of alternative promoters, determining which 5′-most exon is used, as is the case for the mouse α-amylase gene (*15*), for example. Alternatively, there may be post-transcriptional events that are tissue specific or hormone dependent, or both.

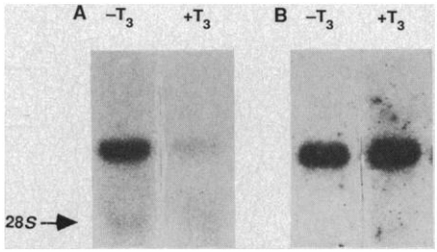

Fig. 4. Effect of T_3 on r-*erbA*β mRNAs in GH_3 cells. Northern analyses of GH_3 cell RNA with (**A**) an r-*erbA*β-2–specific probe and (**B**) an r-*erbA*β-1–specific probe. The probes are the same as those described in the legend to Fig. 3. Total RNA (10 μg) was loaded in each lane. A control probe (β-actin) was used to verify equal loading of RNA per lane. Cells were grown in either medium without thyroid hormone ($-T_3$) (*26*) or in medium supplemented with 10 n*M* T_3 for 24 hours ($+T_3$). RNA was extracted from GH_3 cells by the guanidinium thiocyanate method (*24*).

The high affinity and specificity of r-ErbAβ-2 for T_3 and its similarity in structure with other members of the thyroid and steroid hormone receptor superfamily suggest that r-ErbAβ-2 is a T_3 receptor that could transactivate specific genes in a T_3-dependent manner. Indeed, as is the case with r-ErbAα-1 and r-ErbAβ-1 (*6, 8*), we have shown that r-ErbAβ-2 can function as a T_3 receptor in a model transfection system. The functional significance of the divergent amino termini in the two r-ErbAβs is unclear. Studies with some other members of the steroid and thyroid hormone receptor superfamily suggest that the amino termini of these proteins may act to modulate the transactivation of target genes by the receptor-ligand complex (*16, 17*). For example, two naturally occurring chick progesterone receptors that differ at their amino termini also differ in the magnitude to which they transactivate a hormone-responsive reporter gene (*18*). In our model transfection system, r-ErbAβ-1 and r-ErbAβ-2 appear to function similarly, but it will be of interest to investigate their relative potencies in transactivating the various thyroid-responsive genes in different cell types.

The observation that r-*erbA*β-2 expression is limited to the pituitary gland is intriguing, since that organ has a unique regulatory role in thyroid hormone synthesis through negative regulation of thyroid-stimulating hormone. In addition, the clinical syndromes of isolated pituitary resistance to thyroid hormone (*19*) may be related to a specific receptor deficiency. In this regard, it will be important to determine whether the expression of r-*erbA*β-2 is cell-type specific in the anterior pituitary.

We have previously found that r-*erbA*α-1 and r-*erbA*α-2 mRNAs decrease by approximately 50% after T_3 treatment of GH_3 cells (*5, 13*). It now appears that the similar net reduction in r-*erbA*β mRNA levels (with the common probe) is due to the nearly 90% decrease in r-*erbA*β-2 mRNA coupled with the slight increase in r-*erbA*β-1 mRNA. These findings are in agreement with the previous work of Samuels *et al.* (*20, 21*), who found that a 50% reduction in T_3 receptors in GH_1 cells after T_3 treatment was representative of a heterogenous population of receptors, only some of which were depleted by T_3. It is likely that r-ErbAα-1, r-ErbAα, and r-Erb-Aβ-2 all contribute to the GH_1 cell T_3 receptor pool.

The existence of more than one thyroid hormone receptor species, often within a single tissue, remains of unknown significance. Our demonstration of a tissue-specific and selectively regulated form of thyroid hormone receptor may shed light on the mechanisms underlying the diverse effects of T_3.

REFERENCES AND NOTES

1. H. H. Samuels and J. S. Tsai, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3488 (1973).
2. C. Weinberger *et al.*, *Nature* **324**, 641 (1986).
3. J. Sap *et al.*, *ibid.*, p. 635.
4. C. C. Thompson, C. Weinberger, R. Lebo, R. M. Evans, *Science* **237**, 1610 (1987).
5. M. A. Lazar, R. A. Hodin, D. S. Darling, W. W. Chin, *Mol. Endocrinol.* **2**, 893 (1988).
6. S. Izumo and V. Mahdavi, *Nature* **334**, 539 (1988).
7. T. Mitsuhashi, G. Tennyson, V. Nikodem, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5804 (1988).
8. R. J. Koenig *et al.*, *ibid.* p. 5031.
9. J. Oppenheimer and H. H. Samuels, *The Molecular Basis of Thyroid Hormone Action* (Academic Press, New York, 1983).
10. Analog competition studies were performed in a fashion similar to that described in Fig. 2B. Various amounts of unlabeled analog were used to compete with 0.6 to 0.8 n*M* [^{125}I]T_3.
11. The transfection system is essentially identical to that used in (*8*) except for minor modifications. We used JEG-3 cells rather than COS cells. Also, the CAT reporter gene (pTK14AA) contains two tandem copies of the modified rat growth hormone thyroid response element, rather than the one copy present in pTK14A that was used in (*8*).
12. R. A. Hodin, M. A. Lazar, W. W. Chin, unpublished results.
13. M. A. Lazar and W. W. Chin, *Mol. Endocrinol* **2**, 479 (1988).
14. M. A. Lazar, R. A. Hodin, W. W. Chin, unpublished results.
15. R. A. Young, O. Hagenbuchle, U. Schibler, *Cell* **23**, 451 (1981).
16. S. M. Hollenberg, V. Giguere, P. Segui, R. M. Evans, *ibid.* **49**, 39 (1987).
17. R. Miesfeld, P. J. Godowski, B. A. Maler, K. R. Yamamoto, *Science* **236**, 423 (1987).
18. L. Tora, H. Gronemeyer, B. Turcotte, M. Gaub, P. Chambon, *Nature* **333**, 185 (1988).
19. R. Spanheimer, R. S. Bar, J. C. Hayford, *Arch. Int.*

Med. **142**, 1283 (1982).
20. H. H. Samuels, F. Stanley, L. E. Shapiro, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 3877 (1976).
21. ——, *J. Biol. Chem.* **252**, 6052 (1977).
22. F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977).
23. G. Chamness and W. McGuire, *Steroids* **26**, 538 (1975).
24. J. M. Chirgwin, A. E. Przybla, R. J. MacDonald, W. J. Rutter, *Biochemistry* **18**, 5294 (1980).
25. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
26. H. H. Samuels, F. Stanley, J. Casanova, *Endocrinology* **105**, 80 (1979).
27. We thank H. F. Bunn and J. Majzoub for helpful discussions and S. Tribuna for assistance in preparing the manuscript.

30 August 1988; accepted 3 January 1989

Odor-Induced Membrane Currents in Vertebrate-Olfactory Receptor Neurons

STUART FIRESTEIN* AND FRANK WERBLIN

In olfactory receptor neurons, odor molecules cause a depolarization that leads to action potential generation. Underlying the depolarization is an ionic current that is the earliest electrical event in the transduction process. In two preparations, olfactory receptor neurons were voltage-clamped and stimulated with odors and this generator current was measured. In addition, a method was developed to estimate the time course and absolute concentration of odorants delivered to the receptor sites. With this method, olfactory neurons were found to have relatively high stimulus thresholds, steep dose-response relations, long latencies, and an apparent requirement for cooperativity at one or more steps in the pathway from odorant binding to activation of the generator current.

OLFACTORY TRANSDUCTION IS PRESUMED to be mediated by a membrane-bound receptor protein which is activated and initiates a multistep pathway leading to the generation of action potentials in the olfactory nerve (*1*). An early step in this process is the odorant-induced activation of a depolarizing generator current. Efforts directed toward measurement of this current have been unsuccessful because the small, inaccessible cells have been difficult to impale with microelectrodes. In addition, the indirect access of stimuli to the cells has created conditions in which stimulus parameters such as concentration and duration cannot be known with any surety (*2*). Recently the whole cell patch-clamp technique (*3*) has been applied to isolated olfactory receptor cells with some success, but the absence of a repeatable and enduring odorant response in otherwise healthy cells has been a common disappointment (*4*).

We suspected that the failure of these receptors to give an odor response might have been due to the action of proteolytic enzymes, such as papain, commonly used to isolate the cells from the epithelium (*4*). Accordingly, we developed a slice preparation (*6*) (Fig. 1) that required no enzymatic treatment and left the tissue relatively intact while exposing cells sufficiently to provide access for the patch electrode (*5*).

Because the cells in the slice could respond to odors, we also tried isolating cells from the epithelium by mechanical means that did not require the use of enzymes. With care, viable cells could be isolated and odor responses that were identical to those seen in slice cells were obtained. In approximately 70% of the cells tested, a gigohm seal was attained and successful whole cell recordings obtained. Of these cells nearly 66% showed a response to the odor solution. The only consistent bias in the choice of cells was that cilia be present. There was no difference in the success rate between isolated cells and those in the slice; the results presented here are from cells prepared both ways.

The stimulus was an odorant "cocktail" consisting of acetophenone, amyl acetate, cineole, phenylethylamine, and triethylamine. Saturated aqueous stock solutions of each odorant were prepared in distilled water with a separatory funnel. We could then calculate the molar concentration of each saturated solution from the aqueous saturation coefficients (*7*). These solutions were diluted to 10^{-3} or $10^{-4}M$ with salt solutions that brought the osmolarity to that of the normal Ringer solution. All solutions were brought to *p*H 7.6 with NaOH. Thus the cocktail contained millimolar or 0.1 millimolar concentrations of each of the five odorants in an osmotically balanced Ringer solution.

The current-voltage (*I-V*) relation for the generator current (Fig. 2) was obtained by holding the membrane at potentials from −70 mV to +30 mV and pressure ejecting an odorant pulse (*8*). The current was linear with membrane potential throughout the physiological range and reversed near +5 mV, suggesting that it is a nonspecific cation conductance. The adenosine 3′,5′-monophosphate (cAMP)–activated conductance in ciliary membrane patches shows a similar *I-V* relation (*9*), and the macroscopic current elicited by cAMP application is carried by Na^+, K^+, and Ca^{2+} (*10*).

Odor responses were rarely seen if the concentration of the odorant mixture in the delivery pipette was less than $10^{-3}M$. Initially this seemed high, but with the pressure-ejection delivery system it was impossible to calculate, from pulse duration and pressure alone, the absolute concentration of stimuli reaching the ciliary membrane.

We developed a method of measuring the magnitude of applied odorant at the cilia on the basis of the K^+ permeability of the cell. A stimulus of the odorant cocktail in an elevated (with respect to Ringer solution) K^+ solution was ejected at the cell (Fig 3). An inward current appeared in response to the transient elevation of K^+ and the magnitude of this current was used as a measure of the K^+ concentration reaching the cell (*11*). This value could be used to determine the absolute concentration of odorant at the cell membrane in the following way (*12*). Knowing the concentration of K^+ in the pipette and the concentration arriving at the cell, we were able to determine the fraction of K^+ that reached the cell (K_{frac}) for a given pressure pulse:

$$K_{frac} = [K]_{cell}/[K]_{pipette}$$

where the subscripts cell and pipette mean "at the cell" and "in the delivery pipette," respectively. Because the odorants were mixed to a known concentration in the same pipette solution and the fraction of odorant reaching the cell ($[O]_{cell}$) was the same as K_{frac}, it follows that:

$$[O]_{cell} = K_{frac}\,[O]_{pipette}$$

The subscripts have the same meaning as above.

We took advantage of the finding (Fig. 3) that the change in clamp current to changes in K^+ concentration around the cell was nearly instantaneous, but the onset of the response to odorant was delayed (by the transduction process) by more than 100 ms. Thus the two currents could be measured independently.

Knowing the absolute value of the odor-

Graduate Group in Neurobiology, 275 Cory Hall, University of California at Berkeley, Berkeley, CA 94720.

*To whom all correspondence should be addressed at Section of Neuroanatomy, Yale School of Medicine, 333 Cedar Street, New Haven, CT 06510.