AD-A180 311 RECEPTOR PROTEINS IN VERTEBRATE OLFACTION(U) VIRGINIA COMMONWEALTH UNIV RICHMOND S PRICE 15 OCT 87 ARO-19784.5-LS DAAG29-83-K-0088 1/1

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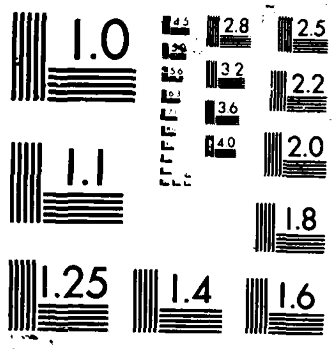
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ARO 19784.5-LS

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TITLE

Receptor Proteins in Vertebrate Olfaction

TYPE OF REPORT (TECHNICAL, FINAL, ETC.)

Final Report

AUTHOR (S)

Steven Price, Ph.D

DATE

October 15, 1987

U. S. ARMY RESEARCH OFFICE

CONTRACT/ GRANT NUMBER

DAAG-29-83-K-0088

INSTITUTION

Virginia Commonwealth University

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S) ARO 19784.5-LS
6a. NAME OF PERFORMING ORGANIZATION Virginia Commonwealth Univ. — 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION U. S. Army Research Office
6c. ADDRESS (City, State, and ZIP Code) 1100 East Marshall Street Richmond, VA 23298	7b. ADDRESS (City, State, and ZIP Code) P. O. Box 12211 Research Triangle Park, NC 27709-2211
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U. S. Army Research Office — 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAAG29-83-K-0088
8c. ADDRESS (City, State, and ZIP Code) P. O. Box 12211 Research Triangle Park, NC 27709-2211	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. / PROJECT NO. / TASK NO. / WORK UNIT ACCESSION NO.

11. TITLE (Include Security Classification)
Receptor Proteins in Vertebrate Olfaction (Unclassified)

12. PERSONAL AUTHOR(S)
Steven Price, Ph.D.

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 6/1/83 TO 5/31/87	1987 Cotober 15	

16. SUPPLEMENTARY NOTATION
The view, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Prior to beginning this project we had developed two hypotheses: (1) that receptor protein in olfactory receptor cells comprise a class of molecules of related structure with a region common to all and a region specific for a particular odorant class; (2) that interaction with receptor proteins is only one of several mechanisms by which olfactory neurons are stimulated by odorants. This project was basically an attempt to test these hypotheses.

Proteins with affinity for compounds related to the odorants, anisole and benzaldehyde, were isolated from extracts of dog olfactory epithelium by affinity chromatography. They are referred to as anisole and benzaldehyde binding protein, respectively. They are not distinguishable by polyacrylamide gel electrophoresis, with molecular weights estimated at 61,000 daltons.

Sera from rabbits immunized with each were general inhibitors of mouse olfactory tissue to odorant stimulation. Monoclonal antibodies were prepared against each protein and

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT ☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code) — 22c. OFFICE SYMBOL

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

tested for ability to alter responses to mouse olfactory epithelium to odorant stimulation. Of the 26 antibodies obtained, 3 (12%) were inhibitors only of the responses to the odorant class bound by the antigen. Presumably, these antibodies are directed against the part of the molecule against which the odorant binds. Another 9 (35%) were inhibitors of responses to all three tested odorants. Presumably, these are directed against the part of the molecule that is accessible (not buried in the cell membrane) and is common to all receptor protein.s The remaining 14 antibodies (54%) were without effect on responses to odorants. Presumably, these are directed against the part of the molecule that is buried in the cell membrane, inaccessible to antibodies. The data are consistent with the hypothesis that olfactory receptor proteins are members of a structurally related class of which the anisole and benzaldehyde binding proteins are members. It is difficult to explain the data in other ways.

None of the antibodies reduced the magnitude of the odorant responses by more than 40%. Therefore, a significant part of the response must be mediated by some pathway other than initiation by binding of the odorant to the receptor protein. This is consistent with the hypothesis that there are multiple mechanisms by which odorants stimulate olfactory neurons, interaction with receptor proteins being one of them.

1. STATEMENT OF THE PROBLEM

Based on work done prior to beginning this project we had developed two hypotheses related to mechanisms of stimulation of olfactory receptor neurons. The first is that olfactory receptor cell responses to odorants can be initiated by a variety of mechanisms, of which binding the odorant by specific receptor proteins is only one. This contrasts with a widely held view that interaction with receptor proteins in cell membranes is the only significant mechanism by which olfactory neurons are stimulated. The second is that olfactory receptor proteins comprise a class of molecules that have structural similarities. That is, that they are in some ways analogous to antibodies, molecules with a large region common to all and a much smaller region in which the antibody specificity resides.

Our approach was to attempt to isolate odorant binding proteins by affinity chromatography (we had already done so with one, anisole binding protein), demonstrate that they were involved in olfactory transduction by preparing antibodies against them and showing that the antibodies inhibited responses of olfactory tissue to odorants (also previously accomplished with anisole binding protein), and attempt to prepare antibodies that were specific inhibitors of the particular odorant class to which the antigen protein had affinity. Such antibodies would constitute nearly unequivocal demonstration that the proteins were not only involved in transduction but were actually the odorant binding molecules through which transduction is initiated.

2. SUMMARY OF MOST IMPORTANT RESULTS

We isolated anisole binding protein from dog olfactory epithelium by the method previously reported and immunized rabbits with it. As had occurred earlier, the rabbit antiserum was a generalized inhibitor of responses to odorants when topically applied to mouse olfactory epithelium. Using a modification of the same procedure we isolated a benzaldehyde binding protein and immunized rabbits against it. This antiserum was also a generalized inhibitor of olfactory responses. This is evidence that both proteins are part of the olfactory transduction system, perhaps as receptor molecules. The antisera cross-reacted with both proteins, showing that there are immunological similarities between them. The proteins had identical behavior on polyacrylamide gel electrophoresis, migrating with apparent molecular weight of 61,000 daltons. However, they are clearly not the same molecule because either could be completely removed from the extract before isolating the other. The immunological cross-reactivity and similar molecular weights are consistent with the hypothesis that they are structurally related molecules.

We prepared monoclonal antibodies against each protein and tested the antibodies' ability to affect responses of mouse olfactory epithelium to odorant stimulation. Of 26 such antibodies, 3 (12%) were specific inhibitors of responses to the odorant class to which the antigen had affinity. That is, one antibody raised against anisole binding protein was a specific inhibitor of responses to anisole and had no significant effect on responses to benzaldehyde or amyl acetate. Two antibodies raised against benzaldehyde binding protein were specific inhibitors of responses to benzaldehyde and were without effect on responses to anisole or amyl acetate. It is difficult to explain the existence of these antibodies unless anisole and benzaldehyde binding proteins are, indeed, receptor molecules for odorants of the anisole and benzaldehyde types, respectively.

Another 9 antibodies (35%) were generalized inhibitors of olfactory responses. That is, they inhibited responses to all three of the test odorants more or less equally. If the antigens are receptor molecules, then these antibodies must be directed against some part of them that is common to both and to other odorant receptor proteins. At least, this is by far the simplest explanation of the data. Obviously, these nine antibodies as well as the three that were specific inhibitors must be directed against a part of the molecule that is not buried in the cell membrane, but is accessible to antibodies on the cell surface.

The remaining 14 antibodies (54%) had no significant effect on responses of mouse olfactory epithelium to odorants. Since the other antibodies raised against the same antigens were inhibitors, it seems reasonable to conclude that these are directed against a portion of the molecule that is buried in the cell membrane and not accessible to antibodies.

We can speculate from the relative occurrances of different types of antibodies that approximately one-tenth of the receptor molecule is the specific odorant binding portion, and that approximately one-half is buried in the cell membrane. Clearly, the data are not adequate for drawing conclusions on these points; they are simply suggestive.

Finally, it was interesting that none of the antibodies reduced responses by more than 40%. That is, at least half the electrophysiological response to odorants was independent of the pathway blocked by these antibodies. We conclude from this that binding to the class of related molefcules exemplified by anisole and benzaldehyde binding proteins is not the only route through which odorants can stimulate olfactory receptor cells. Perhaps there are other receptor proteins of dissimilar structure with which these antibodies do not cross react; perhaps there are other mechanisms not involving receptor proteins at all (as we had hypothesized); perhaps both possibilities are true.

In summary, we have isolated two odorant binding proteins from dog olfactory epithelium. We believe that we have demonstrated that they are olfactory receptor molecules and are representatives of a class of structurally related proteins that includes other olfactory receptor molecules. We have also shown that interaction of odorants with members of this class is not the only mechanism by which olfactory receptor cells are stimulated.

3. PUBLICATIONS PREPARED UNDER SUPPORT OF THIS CONTRACT

S. Price. (1984). Mechanisms of stimulation of olfactory neurons: and essay. CHEM. SENSES 8: 341-354.

S. Price. (1986). Receptor proteins in olfaction. PROC. JAP. SYMP. ON TASTE AND SMELL 20: 8-10.

S. Price. (1987). Receptor proteins in olfaction. BULL. KANAGAWA DENTAL COLL. (in press).

S. Price and A. Willey. (1987). Benzaldehyde binding protein from dog olfactory epithelium. ANN. N.Y. ACAD. SCI. (in press).

S. Price. (1987). Effects of odorant mixtures on olfactory receptor cells. ANN. N.Y. ACAD. SCI. (in press).

4. PERSONNEL PARTICIPATING

Steven Price, Ph.D.
Stephen J. Goldberg, Ph.D.
Sheella Mierson, Ph.D.
Amy Willey, B.S.
Robert E. Revels

None of the personnel earned a degree while employed by the project.

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