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A model system has been used to study the initial electrochemical membrane events in chemoreception by the mammalian olfactory epithelium: Membrane from rat olfactory epithelial homogenates incorporated into planar biomolecular lipid membranes and patch-bilayers.
Data has been obtained to support a hypothesis that the above described system in which it has been demonstrated the presence of ion selective channels coupled with an activated enzyme cascade bears functional relationship to the initial chemoreceptive steps in olfaction.

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FUNCTIONAL RECONSTITUTION OF OLFACTORY RECEPTOR FOR ANALYTICAL APPLICATION.

Final report.

VITALY VODYANOY

May 25, 1988
U.S. ARMY RESEARCH OFFICE

GRANT NUMBER DAAG29-85-K-0109

DEPARTMENT OF PHYSIOLOGY AND BIOPHYSICS,
UNIVERSITY OF CALIFORNIA, IRVINE

A. FOREWORD.

This proposal is the renewal of research grant DAAG29-85-K-0109 entitled "Functional reconstitution of olfactory receptor for analytical application." The present proposal is for continuation of our ongoing research project.

The basic objectives of the project were as the following:

1. Examination of the initial chemoreceptive events in the olfactory system of the rats, using the functional transfer of olfactory receptor membrane from homogenates of the olfactory epithelium into artificial systems.
2. Study of the physical and chemical properties of the chemosensitive ion channels in the reconstituted model system. Investigation of the functional relationship of these ion channels to the olfactory system.
3. Exploration of new approaches to increase the stability and sensitivity of the model chemosensitive system.

The key question of the project was: "How is odorant concentration translated into a functional electrical event in the olfactory receptor cell ?"

Significant progress has been made for most of the objectives of the project.

B. STATEMENT OF THE PROBLEM STUDIED.

We have used a model system to study the initial electrochemical membrane events in chemoreception by the mammalian olfactory epithelium: Membrane from rat olfactory epithelial homogenates incorporated into planar bimolecular lipid membranes and patch-bilayers.

We have obtained data to support our hypothesis that the above described system in which we have demonstrated the presence of ion selective channels coupled with an activated enzyme cascade bears functional relationship to the initial chemoreceptive steps in olfaction.

Chemosensitivity is manifested as a change in the mean open time of single channel events in response to small (subnanomolar) concentrations of the odorants in the medium bathing the membrane under control of the activity of cyclic nucleotide-processing enzymes.

We have studied the kinetics of single channel events associated with the initial steps of olfaction.

Involvement of guanine nucleotide-binding regulatory proteins (G-proteins), of second messenger (c-AMP), and protein kinases have been studied using voltage and patch-clamp techniques together with application of pharmacological agents known to alter the metabolism or effects of the second messenger.

The study of the molecular mechanisms underlying the initial chemoreceptive events together with the development of stable reconstitution system should contribute toward the development of a practical analytical chemosensitive device.

C. SUMMARY OF THE MOST IMPORTANT RESULTS.

This work is concerned with the functional reconstitution of chemosensitive receptors from olfactory epithelium of the rat and also with molecular mechanisms of ion transport associated with olfaction.

We have used three different techniques to transfer the native membrane macromolecules into a model system: (1) Chemosensitive membrane fragments from olfactory cilia were incorporated into bimolecular lipid membranes of large surface area (1 mm^2); (2) Vesicles which contained chemosensitive membrane fragments were attached to the large planar bilayer, and conductance of the membrane system was modified with ion carrier; and (3) The cilia membrane was functionally reconstituted in patch electrode membranes.

We have developed and used an interactive software capable of identifying single-channel transitions in the presence of substantial levels of noise and drift.

We have found and characterized a chemosensitive K+ channel from rat olfactory epithelium homogenates which can be functionally reconstituted into artificial planar lipid bimolecular membranes. We have utilized as a control analogous homogenates from respiratory epithelia, which appear to lack such chemosensitive channels, or at least from which they cannot be reconstituted. The connection of the ion channel which we have characterized with the olfactory system is difficult to rigorously establish, although from the observed absence of the channel in other ciliated epithelial tissues it would appear that it is unique to the olfactory epithelium.

Our data indicate that the steady -state conductance of bilayers modified with olfactory epithelial homogenates became sensitive to very low (subnanomolar) concentration of odorant in the presence of ATP and GTP.

Clustering of single-channel openings was found in presence of cAMP and ATP. We suggest that cyclic gating scheme may result in correlation of successive dwell times, and the irreversible steps included in this cycle may require an energy supply to maintain the steady state.

We have demonstrated that cAMP mimicted the effect of odorant. The statistical analysis of our patch-clamp data suggested the multiple mode of cAMP action on the single channel activity: (a) directly, and (b) via protein kinase system. We hypothesize, that chemosensitivity of functionally reconstituted olfactory receptor is manifested as a change in the mean open time of single channel events in response to small (subnanomolar) concentrations of the odorants in the medium bathing the membrane under control of the activity of cyclic nucleotide-processing enzymes.

Functional transfer of chemosensitive monolayers, membranes and multilayers on to solid substrates can be proposed as a structural basis for development of membrane sensor.

Functional transfer of chemosensitive monolayers, membranes and multilayers on to solid substrates can be proposed as a structural basis for development of membrane sensor.

D. LIST OF PUBLICATIONS ACKNOWLEDGING THE ARO.

1. I. Vodyanoy, V.Vodyanoy and J.K.Lanyi, Biochim. Biophys. Acta, 858, 92-98, 1986.
2. V.Vodyanoy and I.Vodyanoy, Neuroscience Letters, 73, 253-258, 1987
3. V.Vodyanoy, D.Muller, K.Kramer, G.Lynch, and B.Baudry, Neuroscience Letters, 81, 133-138, 1987.
4. V.Vodyanoy and I.Vodyanoy in "Olfaction and Taste", ed.S.Roper, The NY Academy of Sci., NY, 510, 683-685, 1987.
5. I.Vodyanoy, J.E.Hall and V.Vodyanoy, Biophys. J., 53, 649-658, 1987.