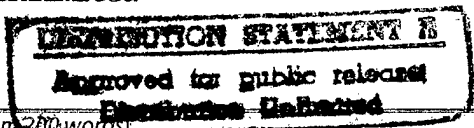


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FINAL PROGRESS REPORT

Grant #: N00014-90-J-1566

R&T Code: 341r002

PRINCIPAL INVESTIGATORS: Barry W. Ache and Robert M. Greenberg

INSTITUTION: University of Florida

GRANT TITLE: Inhibition of olfactory receptor cells: an aquatic model

AWARD PERIOD: January 15, 1993 - December 31, 1995

OBJECTIVE: To develop molecular probes for two second-messenger activated ion channels involved in olfactory transduction, and to use these probes to study how the two signaling pathways are distributed across the ensemble of receptor cells that constitute the olfactory organ.

APPROACH: Molecular biology.

ACCOMPLISHMENTS: In the prior period of support, we showed that cultured lobster olfactory receptor neurons (ORNs) express several types of second messenger-activated ion channels - two activated by IP_3 , one activated by IP_4 and a fourth activated by cAMP. In the present period of support, we initiated a molecular biological effort to begin to develop molecular probes with which to study the distribution of one or more of these ion channels across the receptor cell population. Initial success in cloning a fragment of a cAMP-activated channel from lobster nose RNA could not be sustained in order to obtain the full sequence due to doubts about the authenticity of the fragments. Instead, we pursued the suggested structural similarities between lobster plasma membrane $InsP_3$ -gated channels (IP_3Rs) and known intracellular IP_3Rs in other systems to clone an IP_3R . We amplified a partial cDNA, homologous to known IP_3Rs , from reverse transcribed lobster olfactory organ RNA using degenerate primers and the polymerase chain reaction (PCR). We extended the clone to the 3'-noncoding region using 3'-RACE. A variety of techniques were used to isolate the remaining substantial 5'end, including construction and screening of IP_3R mini-cDNA libraries and 5'-RACE. We have now fully cloned and sequenced cDNAs encoding an IP_3R , as well as a related $G\alpha_q$ protein, from the spiny lobster olfactory organ. The IP_3R cDNA has an 8409 bp open reading frame coding for 2803 amino acids (a computed 320 kDa protein), and is homologous to known IP_3Rs (50-61% amino acid identity). The very rare message has been localized to both the olfactory organ and the brain using an RNase protection assay. The $G\alpha_q$ cDNA has an open reading frame of 1059 bp (353 aa); the sequence is homologous to known $G\alpha_q$ proteins (70-83% amino acid identity). The computed

molecular weight of the protein is 41.5 kDa, consistent with that of the native protein. Northern analysis demonstrates a 4.5 kDa $G\alpha_q$ message in olfactory organ. We are in the process of localizing these molecules to the transduction zone of the cells using antibodies raised to specific sequences.

CONCLUSIONS: Our results provide the basis for developing molecular probes to explore the distribution of two components of the inositol phosphate signaling pathway in lobster olfactory receptor cells.

SIGNIFICANCE: Having multiple effectors in ORNs opens up the interesting and novel possibility that not all ORNs express the same effector(s) and that differences in effector expression may define the existence of functionally different classes of ORNs within the receptor cell population. Such an organizational strategy could have consequence for the design of the detector array in biosensors for odors or other chemical signals.

PATENT INFORMATION: N/A

AWARD INFORMATION: N/A

PUBLICATIONS AND ABSTRACTS:

Publications:

Munger, S.D., Rust, N.R., Wiese, E. and Ache, B.W. Molecular characterization of an olfactory organ G_q protein. (In preparation).

Munger, S.D., Wiese, E., Ache, B.W. and Greenberg, R.M. Molecular and immunocytochemical characterization of an olfactory organ IP_3 receptor. (In preparation).

Abstracts:

Munger, S.D., B.W. Ache and Greenberg, R.M. (1995). Cloning and sequencing of an IP_3 -receptor from lobster olfactory organ. Unpublished Abstract, Gordon Research Conference on Calcium Signals.

Munger, S.D., Ache, B.W. and Greenberg, R.M. (1995). Cloning and sequencing of an IP_3 -receptor cDNA from lobster olfactory organ. Soc. Neurosci. Abstr. 21:1127.

Munger, S.D., B.W. Ache and Greenberg, R.M. (1995). Cloning and sequencing of an IP_3 -receptor partial cDNA from lobster olfactory organ. Chem. Senses. 20:748.

Munger, S.D., Rust, N.C., Ache, B.W. and Greenberg, R.M.
(1996). Cloning and molecular characterization of two
components of the IP₃ pathway from lobster olfactory organ.
Chem. Senses 21:648.