SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 HL 00001-05 LBG

PERIOD COVERED
July 1, 1976 through September 30, 1977

TITLE OF PROJECT (80 characters or less)

Acetylcholine Receptors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Mathew P. Daniels	Staff Fellow	LBG NHLBI
OTHER:	Marshall W. Nirenberg	Chief, Lab. of Biochem. Genetics	LBG NHLBI
	P. Nelson	Chief, Behavioral Biology Branch	BB NICHD
	C. Christian	Special Fellow	BB NICHD
	G. Maloney	NIH Postdoctoral Fellow	LBG NHLBI
	Zvi Vogel	Assistant Professor	Weizmann Institute

COOPERATING UNITS (if any)
Behavioral Biology Branch, NICHD
Neurobiology Unit, Weizmann Institute of Science

LAB/BRANCH
Laboratory of Biochemical Genetics

SE
Section on Molecular Biology

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our aim is to study the distribution of nicotinic acetylcholine receptors in intact and cultured tissues of the peripheral and central nervous system in relationship to the development and function of synapses. To this purpose histochemical localization of α-bungarotoxin bound to the receptors is used in conjunction with light and electron microscopy. In the past year we have used an α-bungarotoxin-horseradish peroxidase conjugate to identify the synaptic sites of nicotinic acetylcholine receptors in the chicken retina and studied the control of nicotinic acetylcholine receptor aggregation on cultured skeletal muscle cells by neuroblastoma-glioma hybrid cells and by substances secreted by these cells.

Project Description:

Methods Employed: We have used indirect immunoperoxidase staining of monolayer cultured cells to which αBT has been bound, and peroxidase staining of tissue incubated in vivo with peroxidase-labeled αBT. These materials are subsequently examined by light and electron microscopy.

Major Findings: (1) Horseradish peroxidase was crosslinked to αBT to form a conjugate which retained the specific affinity of αBT for nicotinic acetylcholine receptors. This conjugate bound to 5-7% of the synapses in the inner synaptic layer of the chicken retina. Amacrine cell and bipolar cell synapses bound conjugate, indicating that some synapses of both types have nicotinic acetylcholine receptors. (2) Co-culture of mouse muscle fibers with neuroblastoma-glioma hybrid cells (which form synapses with the muscle cells) increased the number of nicotinic acetylcholine receptor clusters 2-4 fold. A similar increase was obtained by adding cell-free conditioned medium from hybrid cell cultures to the muscle cell cultures. The effect did not depend on the synthesis of new receptors.

Significance to Biomedical Research: Knowledge of ultrastructural distribution of acetylcholine receptors is of clear importance in any attempt to understand the role of neurotransmitters and their receptors in the function and development of the nervous system. The α-bungarotoxin-immunoperoxidase technique already has shown promise for the diagnosis and analysis of mechanisms in human neuromuscular disorders.

The results obtained with chick retina (1) represent the first direct demonstration of the synaptic localization of neurotransmitter receptors in the central nervous system, and should lead to a better understanding of neuronal specificity in the CNS.

The cultured muscle studies (2) may lead to a better understanding of the mechanism whereby neurons control the distribution of receptors on muscle cells and on other neurons.

Proposed Course: (1) We will extend the study of cholinergic synapses in retina to: a) further analyze the pattern of cholinergic synaptic transmission, b) follow the course of receptor accumulation at synapses during development. (2) We will attempt to characterize the factor(s) in hybrid cell conditioned medium which promotes the aggregation of receptors on muscle cell membranes and to determine its mechanism of action.

Publications:

1. Ringel, S. P., Bender, A. N., Engel, W. K., Daniels, M. P. and Vogel, Z.: A sequential study of denervation-ultrastructural immunoperoxidase localization of alpha-bungarotoxin. Trans. Am. Neurol. Assoc. 100: 52-56, 1975.

2. Bender, A. N., Ringel, S. P., Engel, W. K., Vogel, Z. and Daniels, M. P.: Immunoperoxidase localization of alpha-bungarotoxin (αBT) binding: A new approach to the study of myasthenia gravis. Ann. N. Y. Acad. Sci. 274: 20-30, 1976.

3. Carpenter, David O., Greene, Lloyd A., Shain, William and Vogel, Zvi: Effects of eserine and neostigimine on the interaction of α-bungarotoxin with Aplysia acetylcholine receptors. Mol. Pharmacol. 12: 999-1006, 1976.