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 Z01 HL 00001-05 LBG

PERIOD COVERED
July 1, 1975 through June 30, 1976

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 NHLI
OTHER:	Marshall W. Nirenberg	Chief, Lab. of Biochem. Genetics	LBG NHLI
	P. Nelson	Chief, Behavioral Biology Branch	LBG NHLI
	C. Christian	Special Fellow	BB NICHD
	G. Maloney	NIH Postdoctoral Fellow	LBG NHLI
	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

SECTION
Section on Molecular Biology

INSTITUTE AND LOCATION
NHLI, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

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 studied the ultrastructural distribution of receptors on cultured skeletal muscle fibers and have initiated the following investigations: 1) location and characterization of synapses formed by neuroblastoma hybrid cells in culture 2) modification of histochemical methods in order to permit ultrastructural analysis of receptor distribution in the central nervous system and 3) analysis of the distribution of receptors in the visual system of the goldfish with relationship to optic nerve damage and regeneration.

Project Description:

Objectives: Investigators in this laboratory and others have utilized ^{125}I labelled α-bungarotoxin (αBT) as a label for nicotinic acetylcholine receptors in intact and cultured skeletal muscle, and in embryonic and mature retina. The objectives of this study were to devise a histochemical technique of greater sensitivity and resolution for localizing bound αBT and to apply this technique to studying the ultrastructural distribution of acetylcholine receptors in the peripheral and central nervous system during development, in culture, and in the mature state.

Methods Employed: We have employed indirect immunoperoxidase staining of cryostat sectioned, teased, or monolayered cultured materials to which αBT has been bound. These materials are subsequently examined by light and electron microscopy.

Major Findings: Acetylcholine receptor-rich regions on the surface of muscle fibers grown in culture had previously been observed by light microscope autoradiography with [^{125}I]-labelled αBT. The appearance of these regions could be explained either by (1) the localized presence of complex folds in the plasma membrane or (2) a high local concentration of receptors in the plasma membrane, unrelated to membrane folding. Using the αBT-immunoperoxidase technique with light- and electronmicroscopy we have shown that hypothesis 2 is correct; the plasma membranes of these regions contain at least 7 times the concentration of receptors found in other regions, with no distinctions in cell surface topography.

Significance to Biomedical Research: Knowledge of ultrastructural distribution of acetylcholine receptor 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.

Proposed Course: (1) We are using the αBT-immunoperoxidase technique to help locate and characterize the ultrastructure of synapses which have been detected electrophysiologically in cultures of neuroblastoma hybrid cells with skeletal muscle fibers. (2) We are developing new reagents to adapt the histochemical technique to ultrastrucutral visualization of acetylcholine receptor sites in mature and developing central nervous system tissues. (3) We plan to study the distribution of acetylcholine receptors in the visual system of the goldfish with relationship to the destruction and reformation of synapses during optic nerve degeneration and regeneration.

Publications:

1. Ringel, S. P., Bender, A. N., Festoff, B. W., Engel, W. K., Vogel, Z. and Daniels, M. P.: Ultrastructural demonstration and analytical application of extrajunctional receptors of denervated human and rat skeletal muscle fibres. Nature 255: 730-731, 1975.

2. Vogel, Z. and Daniels, M. P.: The ultrastructure of acetylcholine receptor clusters on cultured muscle fibers. J. Cell Biol. 69: in press.

3. Vogel, Z. and Daniels, M. P.: The acetylcholine receptor of intact and cultured chicken retina cells. Proc. VI Int. Cong. Pharmacol. Vol. 1 Receptors and Cellular Pharmacology. Pergamon Press, New York, 1976, pp. 59-66.