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 00017-04 LBG

PERIOD COVERED
October 1, 1978 - September 30, 1979

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	Research Biologist	LBG NHLBI
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COOPERATING UNITS (if any)

Laboratory of Developmental Neurobiology, NICHD
Neurobiology Unit, Weizmann Institute of Science

LAB/BRANCH
Laboratory of Biochemical Genetics

SECTION
Section on Molecular Biology

INSTITUTE AND LOCATION
NHLBI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
6.5	4.5	2.0

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 continued our study of the formation of cholinergic synapses in developing chick retina, using an α-bungartoxin-horseradish peroxidase conjugate; we have extended our studies on the control of nicotinic acetylcholine receptor aggregation on cultured skeletal muscle cells by macromolecular factors secreted by neuroblastoma-glioma hybrid cells and embryonic neurons; and we have initiated work on the structural interaction between the cytoskeleton and nicotinic acetylcholine receptors in cultured skeletal muscle cells.

PHS-6040
(Rev. 10-76)

Project Description:

Methods Employed: We have used fluorescence staining of monolayer cultured muscle cells with rhodamine-labeled α-bungarotoxin (αBT) and peroxidase staining of tissues incubated in vitro with peroxidase-labeled αBT. These materials are subsequently examined by light or electron microscopy to visualize and quantitate nicotinic acetylcholine receptor sites (AChR).

Ion exchange chromatography, ultrafiltration, and isoelectric focusing have been used to characterize and purify the AChR aggregation factor. Primary cultures of dissociated embryonic neurons and serial cultures of clonal cell lines have been grown as sources of AChR aggregating factor.

^{125}I-αBT binding, detergent treatment and light and electron microscopy have been used to study AChR-cytoskeleton interactions.

Major Findings: An αBT-horseradish peroxidase conjugate was used to study the distribution of AChR (αBT binding sites) in developing chick retina. Incubation of the retina in vitro with the conjugate allowed quantitative comparison of developmental stages. αBT-binding synapses were found at the early stages of synapse formation and comprised between 5 and 11% of the inner plexiform layer synapse population during in ovo development.

The AChR aggregation factor from neuroblastoma x glioma hybrid cells was partially purified by ion exchange chromatography, gel filtration, and preparative isoelectric focusing. Factors with similar activity were detected in embryonic brain and cultures of sympathetic ganglion neurons and spinal cord neurons, but not in liver, adult brain or embryonic glial cell cultures.

Detergent treatment under appropriate conditions removed most lipid and soluble protein from cultured skeletal muscle cells, but left the cytoskeleton and bound components intact. This extraction was used to distinguish tightly bound and loosely bound populations of AChR, which may be correlated with the degree of receptor aggregation.

Significance to Biomedical Research: Knowledge of the 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 results obtained with developing chick retina represent the beginning of an understanding of the role of neurotransmitter receptors in the formation and maturation of chemical synapses, as seen on the ultrastructural level.

The cultured muscle studies 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 of Research:

(1) We have developed a monolayer culture system for physiological and histochemical observation of rabbit retina neurons, which we hope to exploit to learn more about the relationships between αBT binding sites and AChR in central neurons.

(2) We will continue the biochemical characterization of the AChR aggregation factor, adding immunochemical techniques to the array. We will also continue to probe the cellular specificity of factor formation and target receptor specificity of the factor.

(3) We will pursue the study of AChR-cytoskeletal interactions with biochemical and morphological techniques.

Publications:

1) Christian, C.N., Daniels, M.P., Sugiyama, H., Vogel, Z., Jacques, L., and Nelson, G.: A factor from neurons increases the number of acetylcholine receptor aggregates on cultured muscle cells. Proc. Natl. Acad. Sci. USA 75: 4011-4015 (1978)

2) Vogel, Z., Towbin, M., and Daniels, M.P.: α-Bungarotoxin-horseradish peroxidase conjugate: Preparation, properties and utilization for the histochemical detection of receptors to acetylcholine. J. Histochem. Cytochem. 27: 846-851, 1979.