SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 HL 00009-08 LBG

PERIOD COVERED
October 1, 1981 - September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cell Recognition and Synapse Formation

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

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COOPERATING UNITS (if any)

None

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SECTION
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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12	9	3

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)

Monoclonal antibodies were obtained that affect transsynaptic communication, inhibit intercellular adhesion, recognize antigens associated with the synaptic layers of retina, or are expressed by specific cell types in retina such as ganglion neurons, rods and cones, or Muller cells. Five antibodies were obtained that increase the rate of acetylcholine secretion from neuroblastoma-hybrid cells, whereas six antibodies decrease the rate. Another antibody recognizes a 47,000 M_r protein isolated from retina cell membranes, which is distributed in a dorsal-ventral topographic gradient in avian retina. A similar protein also was purified from chick cerebral cortex and thalamus. In addition, monoclonal antibodies were obtained to choline acetyltransferase from rat brain. Evidence was obtained which suggests that acetylcholine of NG108-15 neuroblastoma-glioma hybrid cells is stored in large dense-core vesicles and small clear vesicles and in abnormal large vesicles which lack dense-cores which are produced in a varient cell line.

Project Description: Z01 HL 00009-08 LBG

Objectives:

To identify and characterize molecules involved in synapse formation and function.

Major Findings:

Monoclonal antibodies were obtained that affect transsynaptic communication, inhibit intercellular adhesion, recognize antigens associated with the synaptic layers of retina, or identify certain types of cells in retina. Five antibodies were obtained that bind to antigens that are restricted to the synaptic layers of retina; three of the antigens are restricted to the outer synaptic layer, one to the inner synaptic layer, and one to both the inner and outer synaptic layers of retina. The latter antibody also markedly inhibits intercellular adhesion of retina cells. Two antibodies are specific for retina ganglion neurons, seven for rods and cones, and seven for Muller cells. Other antibodies recognize various sets of neurons; such as horizontal, bipolar, and amacrine neurons of the inner nuclear layer of retina.

Monoclonal antibodies also were obtained that recognize neuroblastoma-hybrid cell antigens. Five antibodies were found that increase the frequency of miniature endplate potentials of myotubes innervated by the hybrid cells; whereas, six antibodies markedly decrease the frequency of miniature endplate potentials. The antibodies that affect rates of acetylcholine secretion from hybrid cells do not alter the cell membrane potential.

In previous studies we showed that a protein in avian retina, termed Top, is distributed in retina in a large dorsal-ventral topographic gradient. The protein thus defines a dorsal-ventral axis of the retina and identifies the relative positions of cells in retina with respect to this axis. During the past year the protein was purified extensively and characterized; the apparent M_r of the protein is 47,000 and the isoelectric point is approximately 4.1. A monoclonal antibody directed against Top in retina also recognizes a protein in chick cerebral cortex and thalamus. The M_r and isoelectric point of the protein isolated from brain were the same as that found for Top from retina.

At this stage, we know relatively little about the regulatory mechanism which relate the number of molecules of the protein detected per cell with the relative positions of cells in retina, except that cells dissociated from retina that were cultured in vitro continue to express the amount of the protein that would be expected based on the original position of the cell in the intact retina. Further studies on both the structure of the protein and the mechanism regulating the expression of the protein are in progress.

The species of transmitter synthesized by a neuron determines, at least in part, the type of neuron and the kinds of synaptic connections that are formed. A project was initiated to serve as a foundation for future studies on

mechanisms that regulate the expression of choline acetyltransferase genes. Choline acetyltransferase from rat brain was purified approxiamtely 100,000-fold and mice then were injected with purified enzyme preparations. Spleen cells from the immunized mice were fused with P3X63 Ag8 myeloma cells and four of the hybridoma cell lines that were obtained were found to sythesize antibodies directed against choline acetyltransferase. Current studies focus on using the antibodies for the purification of mRNA coding for choline acetyltransferase in order to clone the corresponding molecules of cDNA by recombinant DNA methods.

Sixty five monoclonal antibodies were obtained which bind specifically to membrane preparations from NG108-15 neuroblastoma-glioma cells. Six antibodies were found which bind to a greater extent to membranes from differentiated NG108-15 cells than to membranes from undifferentiated NG108-15 cells. Two-dimensional gel electrophoresis also revealed several proteins which are more abundant in membranes from differentiated cells, compared to membranes from undifferentiated cells.

NG108-15 cells contain large, dense-core vesicles and small, clear vesicles. Cells with endogenously synthesized [^{3}H]-acetylcholine were lysed and the vesicles that were released were separated from cell membranes and then fractionated by sucrose density centrifugation. Two well-separated peaks of acetylcholine were found, which comprised greater than 90% of the intracellular [^{3}H]-acetylcholine found. Similar results were obtained with a mutant cell line which lacks large dense-core vesicles, but has large vesicles with amorphous electron lucent cores. These results suggest that acetylcholine in NG108-15 cells is stored in large dense-core vesicles and small clear vesicles and that the clonally inherited change in vesicle morphology in the varient cell line does not affect the ability of the vesicles to store acetylcholine.

Significance to Biomedical Research:

New information was obtained concerning synaptogenesis and synaptic function.

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