DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 HL 00009-10 LBG

PERIOD COVERED

October 1, 1983 - September 30, 1984

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cell Recognition and Synapse Formation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

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LAB/BRANCH

Laboratory of Biochemical Genetics

SECTION

Section of Molecular Biology

INSTITUTE AND LOCATION

NHLBI, NIH, Bethesda, Maryland 20205

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
12	10	2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Undifferentiated NG108-15 neuroblastoma-glioma hybrid cells or NS20-Y neuroblastoma cells can be shifted to a differentiated state by increasing levels of cellular cAMP for several days. cDNA libraries were constructed for species of mRNA that increase in abundance during differentiation. Elevation of cAMP levels of NG108-15 cells for several days results in the expression of new glycoproteins, the disappearance of others, changes in the apparent abundance of some glycoproteins and shifts in the pI of some glycoproteins. The abundance of a voltage-sensitive calcium channel glycoprotein increases during differentiation. A large NG108-15 cDNA library was constructed in λgt11, for use in cloning cDNA for choline acetyltransferase. A monoclonal antibody that recognizes a plasma membrane protein in chick retina cells that is distributed in a dorsal-ventral gradient in retina was injected into chick embryos in ovo. A marked reduction in synapses and neurites in the inner synaptic layer of the retina was observed. cDNA was synthesized from 14 day chick embryo retina RNA and cloned in λgt11 for use in cloning cDNA for the gradient protein. Many monoclonal antibodies were obtained that recognize antigens in the developing nervous system and some of the antigens were characterized. Several antibodies recognize novel gangliosides. A protein was purified from bovine brain that induces neurite outgrowth at nM concentrations and stimulates the phosphorylation of proteins in the absence of Ca^{2+} or cAMP.

Project Description

Objectives

To identify and characterize molecules involved in synapse formation and function and to elucidate mechanisms that regulate gene expression for proteins that are required for synapses.

Major Findings

Populations of relatively undifferentiated NG108-15 neuroblastoma-glioma hybrid cells or NS20-Y neuroblastoma cells can be shifted a more differentiated state by increasing the levels of cellular cAMP for several days. In previous studies the differentiated cells were shown to possess functional voltage sensitive channels for Na^+, K^+, and Ca^{2+}, a Ca^{2+}-dependent K^+ channel that is not voltage-sensitive, long neurites, and small clear vesicles and large dense-core vesicles; whereas, these components were absent or were reduced in undifferentiated cells. Differentiated cells also exhibit higher specific activities of choline acetyltransferase and acetylcholinesterase, secrete more acetylcholine when stimulated, and form more synapses with striated muscle cells than do undifferentiated cells.

During the past year, poly A^+ RNA was obtained from differentiated NG108-15 and NS20-Y cells, cDNA was synthesized and then cloned using plasmid pBR322 as the vector. cDNA corresponding to species of poly A^+ RNA that are more abundant in differentiated cells than in undifferentiated cells were purified by repetitive hybridization with poly A^+ RNA from undifferentiated cells; single-stranded nucleic acids then were separated from double-stranded nucleic acids. The species of cDNA that increase in abundance as cells differentiate were cloned. Some clones will be used as probes to study the mechanisms of cAMP-dependent regulation of mRNA in neuroblastoma cells.

Prolonged elevation of cellular cAMP results in an increase in at least one species of protein that is part of the voltage-sensitive calcium channel complex. Concomittantly, cells acquire functional voltage-sensitive calcium channels. Voltage-sensitive calcium channel proteins were purified extensively. The cDNA libraries that have been constructed will be screened for clones that correspond to the channel proteins.

Our previous analysis of glycoproteins synthesized by NG108-15 cells grown in the presence of PGE_1, an activator of adenylate cyclase, or in the absence of PGE_1, was extended during the past year. The cells were incubated with $[^{35}S]$-methionine for 18 hours, solubilized, and the glycoproteins fractionated by wheat germ agglutinin-, ricin-, or lentil lectin-affinity column chromatography and then by 2-dimensional polyacrylamide gel electrophoresis. ^{35}S-Glycoproteins detected by autoradiography were compared with those detected by silver staining. Both methods of analysis showed that elevation of intracellular cAMP levels for several days results in the expression of new

glycoproteins, the disappearance of others, changes in the apparent abundance of some glycoproteins, and shifts in the pI of some glycoproteins. However, silver staining revealed many glycoproteins that were not detected by autoradiography, including additional glycoproteins that were expressed only by PGE_1-treated cells. These results suggest that elevation of cAMP levels of NG108-15 cells for several days affects the expression of genes for some glycoproteins and alters the post-translational modification of other glycoproteins. Current studies focus on the purification of sufficient amounts of some regulated glycoproteins to obtain partial amino acid sequences of the proteins and to obtain antibodies that recognize the proteins. The cDNA libraries that have been generated will be screened for cDNA that correspond to some regulated species of glycoproteins.

Whether a peripheral neuroblast will give rise to sympathetic or parasympathetic neurons during differentiation is determined by mechanisms that regulate the expression of the genes for tyrosine hydroxylase and choline acetyltransferase, respectively. An extracellular protein and calcium ions are known to be involved in the regulation of these genes, but the mechanisms of regulation are unknown. Previously, choline acetyltransferase from rat brain was purified to essential homogeneity and 4 monoclonal antibodies that recognize the enzyme were obtained. A large NG108-15 cDNA library was constructed using the bacteriophage expression vector, λgt11. Possible cDNA clones that direct the synthesis of choline acetyltransferase in *E. coli* have been detected, but further work is needed to establish the identity of the clones.

A monoclonal antibody was obtained previously that recognizes a large dorsal-ventral concentration gradient of a protein in plasma membranes of chicken retina cells. The amount of protein detected is a function of the position of the cells in retina with respect to the dorsal-ventral axis of the retina. The protein is synthesized by proliferating neuroblasts and by nondividing neurons and the the gradient is formed as the retina is formed. The protein was detected on all cells examined in dorsal and middle retina. Cells that were dissociated from retina and cultured *in vitro* express the amount of gradient protein that would be expected of cells in the intact retina depending upon the original position of the cells in the retina. These results suggest that the gradient is established by an irreversible, clonally inherited mechanism and that once established, the gradient is perpetuated independently by each cell thereafter.

Monoclonal antibody that recognizes the gradient protein, or hybridoma cells synthesizing the antibody, were injected into the amniotic cavity of chick embryos *in ovo* from the second to the fifth day after fertilization and into the vitreal space of chick embryo eyes to determine whether the antibody affects the development or the spatial organization of the retina. The retinas of embryos were continuously exposed to antibody throughout development from the second to the twentieth day after fertilization. Injection of antibody to the gradient protein into the eye resulted in a marked reduction of synapses and neurites in the inner synaptic layer of the retina; whereas, antibodies synthesized by parental P3X63 Ag8 myeloma cells had no effect.

RNA was isolated from 14 day chick embryo retinas and a large cDNA library was constructed in λgt11 that can be used to direct the synthesis of proteins specified by the cDNA in E. coli. The library currently is being screened for recombinants that direct the synthesis of the gradient protein. Injection of poly A^+ RNA from retina into Xenopus laevis oocytes resulted in the synthesis of the gradient protein. This assay can be used for the purification of mRNA for the gradient protein. The cDNA library also is being screened for transducin subunits in collaboration with A. Spiegel.

Seventy-six hybridoma cell lines were generated that synthesize monoclonal antibodies that bind to 8 day chick embryo optic tectum. Fifteen hundred hybridoma lines were generated from spleen cells of mice immunized with the cervical-thoracic spinal cord and dorsal root ganglia of 8 day chick embryos. Some of the hybridoma lines synthesize antibodies that recognize antigens that are restricted to fiber tracts or neuronal cell body regions of the spinal cord.

Additional information was obtained about other antigens that are recognized by monoclonal antibodies. For example, antigen 13H9 was shown to be a protein with an approximate M_r of 180,000. The antigen is associated with cell membranes of all chick retina cells but has not been detected on neurons or glia in other parts of the nervous system. The antigen defines a functional set of cells in the nervous system.

18B8 antigens are first expressed by ganglion neurons and then by other types of neurons in retina. The antigens are found on cell soma initially, but later in development antigens disappear from cell soma and can be seen in a highly stratified, multi-laminar pattern in the inner synaptic layer of the retina and in a circular "organelle" in the outer synaptic layer. The antigens are expressed by approximately 10% of the cells in retina. In collaboration with Victor Ginsburg and his colleagues, the antigens were shown to be novel gangliosides of unknown structure that contain disialyl residues whose abundance and structure change during development; the location of the gangliosides in retina also changes during retinal development. Most of the antigens are associated with the inner and outer synaptic layers of retina in late embryo and adult retina. In addition, the antigens for many other monoclonal antibodies were characterized and in some cases were partially purified.

A heat-stable, acidic, soluble, bovine brain protein was found that induces neurite outgrowth from chick embryo cerebral cortical neurons at nM concentrations in defined medium. The Neurite Extension Factor (NEF) rapidly stimulates the phosphorylation of a protein with an apparent Mr of 90,000 in the absence of calcium ions or cyclic nucleotides. Phosphopeptide mapping results show that the 90,000 Mr protein is related to an 87,000 Mr protein that is a major substrate for C kinase in brain.

Significance to Biomedical Research

New information was obtained concerning synaptogenesis and synaptic functions.

Publications

1. Nirenberg, M., Wilson, S. P., Higashida, H., Rotter, A., Kreuger, K., Busis, N., Ray, R., Kenimer, J., Adler, M., and Fukui, H.: Synapse Formation by Neuroblastoma Hybrid Cells. In: Molecular Neurobiology. Cold Spring Harbor Symposia on Quantitative Biology XLVIII: 707-715. (1983).

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3. de Blas, A., Adler, M., Shih, M., Chiang, P. K., Cantoni, G. L., and Nirenberg, M.: Inhibitors of CDP-choline synthesis, action potential calcium channels, and stimulus-secretion coupling. Proc. Natl. Acad. Sci. 81: 4353-4357. (1984).

4. Kligman, D.: Neurite outgrowth from cerebral cortical neurons is promoted by medium conditioned over heart cells. J. Neurosci. Res. 8, 281-287, (1982).

5. Trisler, D., Grunwald, G.B., Moskal, J., Darveniza, P., and Nirenberg, M.: Molecules that identify cell types or position in the retina. In: Neuroimmunology. Behan, P.O. and Spreafico, F. (Eds.). New York, Raven Press, 89-97. (1984).

6. Nirenberg, M., Krueger, K., Rotter, A., Wilson, S., and Higashida, H.: Regulation of Synapse Formation by Cyclic AMP: In: The Symposium of the International Society for Developmental Neurosciences. In Press.

7. Fredman, P., Magnani, J. L., Nirenberg, M., and Ginsburg, V.: Monoclonal antibody A2B5 reacts with many gangliosides in neuronal tissue. Archives of Biochemistry and Biophysics. In Press.

8. Strauss, W. L. and Nirenberg, M.: Inhibition of choline acetyltransferase by monoclonal antibodies. Journal of Neuroscience. In Press.