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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 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 the regulated

glycoproteins to obtain partial amino acid sequences for the proteins and to obtain antibodies that recognize the proteins. The cDNA libraries that have been generated will be screened for cDNA that corresponds 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 thereafter, the gradient is perpetuated independently by each cell.

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 M_r of 90,000 in the absence of calcium ions or cyclic nucleotides. Phosphopeptide mapping results show that the 90,000 M_r protein is related to an 87,000 M_r protein that is a major substrate for C kinase in brain.

Further information has been obtained on the aggregation of nicotinic acetylcholine receptors on cultured myotubes induced by neuronal factors. In experiments using image intensification to directly observe changes in receptor distribution, and electron microscopy to study changes in the subsurface cytoskeleton and extra-cellular matrix, we have demonstrated discrete steps in the assembly of receptor aggregates from diffuse receptors. The transition from microaggregates of acetylcholine receptors, which appear first, to large dense aggregates is dependent on temperature and involves an increase in the stability of the aggregate.

The fine structure was studied of regions of myotubes containing microaggregates of nicotinic acetylcholine receptors that form within 90 minutes of exposure to embryonic brain extract. A mixture of rhodamine conjugated α-bungarotoxin and peroxidase conjugated toxin was used so that the formation of receptor microaggregates could be observed directly and so that the distribution of acetylcholine receptors on myotube membranes also could be determined. Small receptor aggregates are converted to larger aggregates that are more stable than the smaller ones. Microaggregates form in the presence of embryonic brain extract and accumulate at temperatures between 18° and 23° but do not form larger aggregates. At 36° C aggregates rapidly form from microaggregates. Microaggregates are destabilized rapidly when brain extract is removed or when sodium azide is added to inhibit ATP formation. In contrast, aggregates remain stable for several hours under these conditions. Azide reversibly blocks the formation of both microaggregates and aggregates at 36° C. Electron micrographs of microaggregates reveal characteristic mounds in the cell surface, subtended by loosely organized cytoplasmic filaments. The receptor aggregates have, in addition, an increased association with basal lamina and a characteristic dense filamentous structure below the cell membrane.

The regulation of the gene coding for preproenkephalin, the precursor of the opioid peptides methionine-and leucine-enkephalin, was investigated. cDNA was cloned in the Pst I site of pBR322. A full-length cDNA clone corresponding to rat striatum preproenkephalin mRNA was found and sequenced. The primary structure of the rat preproenkephalin protein deduced from the nucleotide sequence of the cDNA (269 amino acid residues, M_r 30932) is similar to bovine and human preproenkephalin (78% and 82% matched amino acid residues, respectively) and contains 4 copies of Met-enkephalin, 1 of Leu-enkephalin, 1 of Met-enkephalin-Arg-Gly-Leu, and 1 of Met-enkephalin-Arg-Phe. Southern blot analysis of rat genomic DNA with a probe prepared from the rat preproenkephalin clone suggests that the rat contains a single gene for preproenkephalin. The relative abundances of rat preproenkephalin mRNA are as follows: striatum 100, hypothalamus 11.2, pons + medulla 10.8, spinal cord 10.3, cerebellum 6.1, midbrain 5.9, frontal cortex 4.6, hippocampus 2.0, thalamus 1.6. Electroconvulsive shock treatment (1 sec per day) of rats for 10 days elicited increases of 78% and 0-14% in the relative abundances of preproenkephalin mRNA of the hypothalamus and striatum, respectively.

Preproenkephalin mRNA was detected in NG108-15 mouse neuroblastoma x rat glioma poly A^+ RNA by Northern blot hybridization. The abundance was 1/650 of that of the rat striatum and was increased 3.5 fold by treatment of the cells with the glucocorticoid hormone, dexamethasone, for 4 days. This cell line can be used for the study of preproenkephalin gene expression.

Large cDNA libraries were prepared from cat dorsal root ganglion poly A^+ RNA and rat spinal cord poly A^+ RNA, for the isolation of clones containing cDNA for the precursors of tachykinin neuropeptides.

Histidyl-proline diketopiperazine (cyclo(His-Pro)), a metabolite of the thyrotropin releasing hormone, has been reported to inhibit prolactin secretion from the pituitary, elicit anti-depressant effects, alter cyclic nucleotide

levels, and alter body temperatures. A search for receptors for cyclo(His-Pro) revealed specific binding sites for cyclo(His-Pro) in particulate fractions derived from bovine adrenal cortex or liver, but not in fractions derived from brain or pituitary. A single class of binding sites was found with a K_d of 900 nM and a maximum number of sites of 92 pmol per mg protein. The binding was stereospecific and the histidine moiety of the peptide was a major determinant of the binding. The binding sites for cyclo(His-Pro) were inactivated by incubation of particulate fractions with trypsin or at 100° C. No metabolism of cyclo(His-Pro) was detected.

Some strains of E. coli accumulate toxic levels of methylglyoxal that inhibit cell growth. One such strain was isolated and shown to synthesize a mutant form of the cAMP receptor protein and to lack the gene for adenylate cyclase. Growth of the cells on glucose-6-phosphate, but not glucose, resulted in premature growth arrest due to the accumulation of methylglyoxal. The specific activity of phosphofructokinase in the mutant cells was elevated. The mechanism of growth arrest in the mutant cells was suggested to involve an increase in the synthesis of triose phosphate via glycolysis with spillover of metabolites into a pathway leading to the formation of methylglyoxal.