med all projects reports

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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 which 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 a large dorsal-ventral 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; 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 protein in brain was purified and characterized; the M_r and isoelectric point of the protein from brain were the same as that found for Top from retina. At this stage, we know relatively little about the regulatory mechanism which relates the number of molecules of the protein detected per cell with the relative positions of the cell in the 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 approximately 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; four of the hybridoma cell lines that were obtained were found to synthesize antibodies directed against choline acetyltransferase. The current objective is to use 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.

We previously showed that the acquisition of functional voltage-sensitive calcium channels by NG108-15 cells enables the cells to form synapses with striated muscle cells. A 1,4-dihydropyridine analog, [^{3}H]-nitrendipine, which reportedly binds specifically to voltage-sensitive calcium channels, was used as a probe for voltage-sensitive calcium channels of NG108-15 cells. [^{3}H]-Nitrendipine was shown to bind specifically to membranes prepared from differentiated NG108-15 cells; the dissociation constant, determined by Scatchard analysis, was approximately 2×10^{-10} M. The number of specific binding sites for [^{3}H]-nitrendipine per average cell was shown to increase 10-fold, from 1,600 to 16,000, when cells were treated for 3 or more days with PGE_1 which activates adenylate cyclase and elevates cellular cAMP levels. These results suggest that prolonged elevation of cellular cAMP levels results in an increase in the number of voltage-sensitive calcium channels per cell and that the ability of the cells to form synapses is regulated by the rate of synthesis and/or turnover of voltage-sensitive calcium channels.

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.

A protein was obtained from bovine brain which markedly stimulates neurite outgrowth by cultured neurons dissociated from chick embryo cerebral cortex. The protein was purified approximately 200-fold. The native protein is a dimer with an apparent M_r of 75,000 which on reduction dissociates into subunits with an M_r of 37,000. The protein, estimated to be $>$ 90% pure, induces neurite outgrowth at a concentration of approximately 1 nM. A rabbit antiserum to the neurite extension factor, termed NEF, was obtained which blocks the activity of the protein in stimulating neurite extension. Neurite extension protein was detected by immunohistochemical methods in neurons in some, but not all, regions of adult rat brain. These results suggest that NEF is required for neurite extension by some neurons in the central nervous system.

NG108-15 cells release a protein into the medium which induces the formation of clusters of nicotinic acetylcholine receptors on myotube plasma membranes. Laminin, a myotube basement membrane glycoprotein, was shown to potentiate the aggregation of the acetylcholine receptors. Basement membrane proteins of myotubes such as laminin, collagen types IV and V, and heparin

sulfate proteoglycan were detected on the surface of muscle fibers at early stages of receptor aggregation in vivo. The basement membrane proteins associated with acetylcholine receptor aggregates were relatively resistant to extraction by detergent. Exposure of myotubes to brain extracts resulted in marked aggregation of acetylcholine receptors on myotubes and the appearance of cytoskeletal specializations under myotube plasma membranes. These results suggest that the binding of laminin to the muscle cell surface and the formation of cytoskeletal structures beneath the cell membrane may promote the formation of nicotinic acetylcholine receptor aggregates and/or stabilize the receptor aggregates.

Polyadenylated mRNA was purified from adrenal medulla and brain and was used to direct the cell-free synthesis of preproenkephalin, a precursor of opioid enkephalin pentapeptides. The apparent M_r of preproenkephalin was found to be 30,000. The bovine precursor was shown to contain the amino acid sequences of both methionine- and leucine-enkephalin. Preproenkephalin was converted to a smaller protein, termed proenkephalin, with an apparent M_r of 28,000. Monoclonal antibodies directed against Met-enkephalin and Met-enkephalin [ArgPhe] were obtained. A rat brain cDNA library was prepared by recombinant DNA techniques which will be screened for rat preproenkephalin clones and other clones of interest. cDNA hybridization probes then will be used to study the regulation of enkephalin gene expression. In a related project, corticotropin-releasing factor was shown to stimulate the secretion of beta-endorphin and corticotropin from clonal AtT-20 mouse pituitary tumor cells and to increase the activity of adenylate cyclase of the cells.

Histidyl-proline diketopiperazine, after injection into rats, was cleared from the circulation with biphasic kinetics ($t_{1/2}$ = 1.25 and 33 min). Unmetabolized histidyl-proline diketopiperazine appeared rapidly in the urine. The longer half-time of clearance of the peptide of 33 min suggested a tissue reservoir of the peptide. Histidyl-proline diketopiperazine was found to accumulate in adrenal, liver, and kidney. Therefore, specific binding of the diketopiperazine to membrane preparations was examined. Such binding was observed in membrane preparations derived from adrenal and liver.