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Two λgt11 cDNA libraries from human brain were screened with 3 oligodeoxynucleotide probes for recombinants coding for α subunits of G signal transducing proteins, which couple receptors activated by hormones or light to effectors such as adenylate cyclase or cGMP phosphodiesterase. Fourteen of the 500,000 recombinant clones screened from a human basal ganglia cDNA library and 12 of the 400,000 clones screened from a human cerebral cortex library were detected with 2 or 3 of the ^{32}P-probes used. DNA inserts from 13 positive clones were sequenced partially; 11 clones were identified as α_s cDNA and 2 clones as α_i. The DNA insert from one of the α_s clones was sequenced completely and additional partial sequences were obtained for 10 α_s clones. Four species of α_s cDNA were found that differ in nucleotide sequence in the region that corresponds to α_s amino acid residues 71-88. The clones differ in the codon for α_s amino acid residue 71 (glutamic acid vs. aspartic acid), the presence or absence of codons for the next 15 amino acid residues, and the presence or absence of an adjacent serine residue. A mechanism was proposed for generating 4 species of α_s mRNA by alternative splicing of precursor RNA transcribed from a single gene.

cDNA from one of the two human α_i clones was sequenced completely (BG-4), and a partial sequence was obtained for the second clone. The first nucleotide residue of BG-4 α_i cDNA corresponds to the 14th residue of the bovine α_i coding sequence and the last residue of BG-4 (1261) is in the 3'-untranslated region. The amino acid sequence derived from the nucleotide sequence of human BG-4 α_i cDNA is highly homologous to bovine and rat α_i sequences reported by others. In addition, the 3'-untranslated region of BG-4 α_i cDNA is highly homologous to the 3'-untranslated regions of bovine and rat α_i cDNA. The 3'-untranslated nucleotide sequences of human, bovine, and rat α_s cDNAs also are highly conserved, but differ markedly from α_i 3'-untranslated sequences. These results suggest that the 3'-untranslated regions of α_s and α_i genes and/or mRNA are needed for functions that have not been identified thus far.

In previous studies we have shown that elevation of cAMP levels of NG108-15 neuroblastoma-glioma hybrid cells or neuroblastoma cells for several days results in 10-100 fold increases in the activity of voltage-sensitive calcium channels, 15-45 fold increases in spontaneous secretion of acetylcholine at synapses, and 5-15 fold increases in the abundance of synapses with cultured striated muscle cells. In addition, the number of molecules of the voltage-sensitive calcium channel protein subunit that binds [^{3}H]-nitrendipine increases 12-fold. We previously obtained about 100 cDNA clones that hybridize to species of mRNA that are more abundant in NG108-15 or NS20-Y cells that had been treated with dibutyryl cAMP for several days then in untreated control cells. Quantitative studies on the extent of increase in abundance of the species of mRNA that respond to dibutyryl cAMP were performed using the cloned cDNA as probes. Twenty cDNA clones were obtained that hybridize to species of poly A$^+$ RNA that increase in abundance 10-90 fold due to treatment of cells with dibutyryl cAMP. Northern blots also were performed and the number of bands of poly A$^+$ RNA that hybridize to each cloned cDNA probe and their chain lengths were determined.

Affinity purified antibodies to the α, β, and Y protein subunits of voltage-sensitive calcium channels were used to screen a λgt11 cDNA library prepared from poly A^+ RNA from rat skeletal muscle. Approximately 20 recombinant clones were found that were identified tentatively as calcium channel α subunit cDNAs. Other cDNA clones were obtained that are putative Y subunit clones.

In previous studies a putative cDNA clone for choline acetyltransferase was found. We now have determined the nucleotide sequence of the 1118 bp DNA insert. Partial amino acid sequences of several peptides derived from choline acetyltransferase by the action of peptidases were obtained in collaborative studies by Lou Hirsh and his colleagues in Dallas. The λgt11 cDNA library was screened again with 2 new oligodeoxynucleotide probes to different regions of choline acetyltransferase and cDNA clones were obtained that were recognized by both probes. Further studies with these clones are in progress.

Antigenic molecules termed TOP, which are distributed in a dorsal > ventral concentration gradient in chicken retina, are expressed early in development (by 48 hr after fertilization) in the optic cup of chicken embryos and continue to be expressed in retina thereafter. ^{35}S-labeled-TOP-antibody complexes were purified by protein A-Sepharose column chromatography and subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis and autoradiography. TOP also was purified from dorsal retina by anti-TOP IgG-Affigel 10 affinity column chromatography. Both purification methods yielded one major band of protein with an M_r of approximately 47,000. A protein of M_r approximately 47,000 also was purified from chicken embryo brain. Cultured cells dissociated from 8-day chicken embryo retinas accumulated the amount of TOP expected of cells in the intact retina, depending on the position of the cells in the retina. TOP accumulations by cells dissociated from dorsal or ventral retina, mixed in different proportions and cocultured were additive. These results show that TOP is a protein, that the gradient of TOP is established early in development, and that perpetuation of the gradient does not depend on the continuous presence of an extracellular gradient of diffusable molecules or on maintenance of interactions between cells. Synapses and neurites in the retina of developing chick embryos were reduced markedly by injection of anti-TOP antibody into the eye.

The addition of bradykinin to NG108-15 cells was shown in previous studies to increase cellular levels of inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol. The newly synthesized IP_3 in turn stimulates the release of stored calcium ions into the cytoplasm, thereby activating calcium-dependent K^+ channels. The increased efflux of K^+ ions results in cell hyperpolarization. This is followed by cell depolarization due to inhibition of M channels, thereby decreasing the rate of K^+ efflux from cells via M channels. Additional results now show that inhibition of M channels is due to diacylglycerol and Ca^{2+} dependent activation of protein kinase C. Several phosphoproteins were detected by two dimensional gel electrophoresis whose synthesis is dependent upon the addition of bradykinin to cells. Whereas, injection of inositol 1,4,5-trisphosphate inside NG108-15 cells results in the release of stored calcium into the cytoplasm, injection of inositol 1,3,4-trisphosphate or inositol 1,3,4,5-tetrakisphosphate has little or no effect on calcium mobilization, but instead results in the activation of nonspecific cation channels. Calcium ions are not required for the activation of the nonspecific

cation channels. The nature and significance of these findings warrant further investigation in light of recent reports that inositol 1,3,4-trisphosphate and inositol 1,3,4,5-tetrakisphosphate are present in some tissues and that inositol 1,3,4,5-tetrakisphosphate is synthesized by phosphorylation of inositol 1,4,5-trisphosphate, catalyzed by an appropriate kinase, and that inositol 1,3,4-trisphosphate is formed by dephosphorylation of inositol 1,3,4,5-tetrakisphosphate.

Immunofluorescence staining on cryostat sections prepared from embryonic brain extract-treated myotubes revealed a precise colocalization of a 43,000 M_r cytoplasmic protein (distinct from actin) with newly-formed ACh receptor aggregates. This result is consistent with a role for the 43,000 M_r protein in receptor immobilization, as suggested indirectly by studies from other laboratories on fish electric organ and the neuromuscular junction.

We previously showed that partially purified and highly purified fractions from the extracellular matrix of the Torpedo electric organ induce ACh receptor aggregation in cultured myotubes with a time course similar to that of embryonic pig brain extract. We now have found that antiserum against a partially purified fraction from Torpedo (700 units/mg protein) can absorb about 60% of the receptor aggregation activity of brain extract. Under the same conditions, 90% of the activity in the Torpedo fraction was absorbed. This result is consistent with the presence of immunologically related aggregation factors in electric organ and brain.

We previously showed that neural factor induced formation of ACh receptor aggregates on tetrodotoxin-treated myotubes is associated with the localized deposition of basal lamina. We now find that embryonic brain extract and ciliary ganglion explants induce a widespread deposition of basal lamina on non-tetrodotoxin-treated myotubes. Ascorbate oxidase blocks this deposition of basal lamina, suggesting that ciliary ganglion and embryonic brain extract contain ascorbate-like factors that promote muscle basal lamina formation. The extensive induction of ACh receptor aggregates by ciliary ganglion explants was only partially inhibited by ascorbate oxidase, and basal lamina deposition still occurred at the ACh receptor aggregate sites. These results suggest that the ascorbate-like factor contributes to, but is not primarily responsible for the induction of receptor aggregates. In addition, they suggest that deposition of basal lamina at receptor aggregates can occur independently of the ascorbate-like factor.

We have been studying hormonal and neurotransmitter-dependent mechanisms that regulate the gene for proenkephalin (pEnk), the precursor of the opioid peptides methionine- and leucine-enkephalin, in clonal cell lines of neural origin, as well as in rat brain. NG108-15 neuroblastoma-glioma hybrid cells and C6 rat glioma cells contain pEnk mRNA, quantitated by blot hybridization. C6 cells contain a much higher abundance (3-6 pg/μg RNA) but lower enkephalin content than NG108-15 cells. Treatment of C6 cells with compounds that activate adenylate cyclase and raise the cAMP concentration (e.g. by a beta-adrenergic receptor agonist such as (-)-norepinephrine or by forskolin) elevate the pEnk mRNA abundance. Glucocorticoid hormones such as dexamethasone or cortisol, while having no effect alone on the pEnk mRNA level, potentiate the effect of cAMP elevation, producing maximum elevations of 8-fold. C6 cells contain proenkephalin but do not process this precursor significantly. Treatment with

norepinephrine and dexamethasone raises the content of proenkephalin 11-fold. Treatment of cells with glucocorticoid and forskolin for 1-6 hr increases pEnk gene transcription at least 2.5-fold. These results suggest that glucocorticoids and neurotransmitters that elevate cAMP transcriptionally regulate enkephalin biosynthesis in enkephalinergic cells.

Studies have been initiated on the regulation of expression of the gene for proneuropeptide Y (pNPY), the precursor of neuropeptide Y, a putative regulator in the autonomic nervous system. pNPY mRNA is relatively abundant in NG108-15 hybrid cells. Treatment of these cells with glucocorticoids elevates pNPY mRNA 2-fold.

Two novel neuropeptides having anti-analgesic activity were recently isolated and sequenced by Dr. H. Y. Yang's group. Their structures are Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-NH_2 (A18F-NH_2) and Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2. A rat hypothalamus λgt11 cDNA library was screened with ^{32}P-oligodeoxynucleotides corresponding to portions of these peptides and putative A18F-NH_2 cDNA clones were obtained.

Nearly all prokaryotic genes use the translation initiation codon AUG. However, there are a few examples where GUG or UUG function as initiation codons in E. coli. The gene for E. coli adenylate cyclase, is one of the genes that uses the unusual UUG initiation codon. We have investigated the effect of this unusual initiation codon on the expression of the adenylate cyclase gene by changing the DNA sequence coding for the UUG initiation codon to ATG and GTG, using oligonucleotide-directed mutagenesis. A comparison of the activities associated with the three codons was made in three different environments: (1) in the normal environment, with the adenylate cyclase gene expressed from its own promoters, (2) in a transcription fusion with the adenylate cyclase gene under the transcriptional control of the phage lambda promoter, and (3) in a gene fusion with the adenylate cyclase gene fused to the E. coli galactokinase gene to generate a fusion protein with galactokinase activity. In each of the three environments, it was observed that the UUG initiation codon had the lowest efficiency of translation initiation and the AUG initiation codon had the highest efficiency, while the GUG initiation codon was intermediate. These results may provide a partial explanation for the finding that the cellular concentration of adenylate cyclase is very low.

In E. coli cAMP plays a crucial role in regulating the expression of inducible genes. The levels of this nucleotide are controlled primarily by a catabolite-dependent modulation of adenylate cyclase activity. Insight into the mechanism of regulation of the activity of this enzyme has come primarily from studies of permeable cells. Current information suggests that the phosphoenolpyruvate:glucose phosphotransferase system (PTS) is intimately involved in the regulation. Additionally, potassium and phosphate ions play key roles in modulating adenylate cyclase activity. A model for interaction of adenylate cyclase with PTS proteins and potassium phosphate to form a regulatory complex was proposed previously by us. The purpose of the present study was to test the proposed model for adenylate cyclase regulation using a reconstitution approach. We found that all of the unique features of adenylate cyclase characteristic of the regulatory complex observed in permeable cells were reconstituted in cell-free extracts. The results strongly support the proposal that adenylate cyclase activity is regulated by PTS proteins.