NEUROTROPHIC FACTORS

Organizers: Ralph Bradshaw and Dennis Cunningham

April 17-23, 1990

Plenary Sessions Page

April 18:
- Neurodevelopment (joint)....2
- Biosynthesis of Neurotrophic/Growth Factors....80

April 19:
- Molecular Analyses of Channels and Receptors (joint)....4
- Integrins and Cell Surface Receptors for Neurotrophic Factors....81

April 20:
- Learning/Plasticity (joint)....7
- Neuronal Responses to Neurotrophic Factors-I....82

April 21:
- Intracellular Communication (joint)....9
- Neurotrophic Factors in Neurodegenerative Disease/Regeneration....84

April 22:
- Intercellular Communication (joint)....14
- Neuronal Responses to Neurotrophic Factors-II....85

Poster Sessions

April 18:
- Neurotrophic and Growth Factors (CR 100-116)....87

April 19:
- Neuronal Responses (CR 200-213)....92

April 20:
- Disease and Regeneration (CR 300-307)....97

April 22:
- Glial Cells (CR 400-408)....100

Neurotrophic Factors

Biosynthesis of Neurotrophic/Growth Factors

CR 001 BIOSYNTHESIS OF EGF AND NGF, Ralph A. Bradshaw, Michael Blabber, and Paul J. Isackson, Dept. of Biological Chemistry and Dept. of Anatomy and Nurobiology, College of Medicine, University of California, Irvine, California 92717

Most polypeptide growth factors and hormones are elaborated as high molecular weight precursor structures. These proteins, often arising from multiple transcripts from the same gene, are processed by proteolytic enzymes, often differentially, giving rise to different forms of the active agents in different organisms and tissues. Thus, the regulation of a specific hormonal response begins at the transcriptional level and precedes through post-ribosomal modifications, which may include other covalent changes in addition to limited proteoalysis.

The processing of the precursors of NGF and EGF may in part be controlled by the kallikreins associated in non-covalent high molecular weight complexes that are characteristic for these hormones. Epidermal growth factor binding protein (EGF-BP) and the gamma subunit of nerve growth factor (gamma NGF) are highly similar in sequence at both the protein (85%) and cDNA (92%) levels. They can, however, be distinguished by their highly specific affinities for EGF and beta NGF, respectively, by their catalytic constants for the hydrolysis of a number of synthetic substrates and by the limited protealysis that they undergo themselves. Using site-directed mutagenesis to generate a variety of chimeric molecules (in which various segments of the enzymes have been interchanged) we have determined the role of critical residues (or regions) that explain the differences in catalytic specificity and growth factor association. These findings underscore the possibility that even small changes in members of a relatively standard enzyme family can provide a high degree of selectivity for the purposes of specific precursor processing. (Supported by U.S. Public Health Service, NS 19964, and American Cancer Society, BC 273.)

CR 002 REGULATION OF GLIA-DERIVED NEXIN, Denis Monard, Melitta Dihanich, Angelo Guidolin, Marie Charlotte Hoffmann*, Eva Reinhard, Georgio Rovelli, Juerg Sommer and Cordula Nitsch*. *Department of Anatomy, University of Basel, and Friedrich Miescher Institute, P.O. Box 2543, 4002 Basel, Switzerland.

The glia-derived nexin (GDN) is a 43 kDa serine protease inhibitor originally isolated for its neurite-promoting activity in cultures of neuroblastoma cells [1]. GDN also promotes neurite outgrowth in dissociated primary neuronal cultures of the chick sympathetic ganglion and of the rat hippocampus [2,3]. *In vivo*, GDN is constitutively expressed in the olfactory system, a structure where neuronal degeneration and regeneration take place throughout life [4]. GDN synthesis, which is normally down-regulated in the sciatic nerve, increases during the days following a lesion [5]. In the gerbil, immunocytochemistry demonstrates an increase of GDN-positive cells in the hippocampal CA1 region where pyramidal cell death has been specifically caused by ischemia. The amino acid sequence of rat and human GDN has been established through cDNA cloning [6,7]. The sequence of protease nexin 1 derived from human fibroblasts is identical to that of human GDN [8]. Elastase cleavage between the P1 and P2 residues leads to an inactive protein, thus demonstrating the importance of the reactive center [9]. Heparin strongly potentiates the complex formation between GDN and thrombin. The complex thrombin-GDN binds to vitronectin with an apparent dissociation constant of 1.7 nM. A similar interaction is measured with laminin. Thrombin and GDN modulate neurite outgrowth on permissive substrates such as vitronectin and laminin. Deletion analysis and site-directed mutagenesis have been used to better define the interaction of GDN with heparin and the importance of the three cysteine residues.

[1] Guenther et al., EMBO J. 4, 1963 (1985); [2] Zurn et al., Dev. Neurosci. 10, 17 (1988); [3] Farmer et al. Dev. Neurosci., in press; [4] Reinhard et al., Neuron 1, 387 (1988); [5] Meier et al., Nature 342, 548 (1989); [6] Gloor et al., Cell 47, 687 (1986); [7] Sommer et al., Biochemistry 26, 6407 (1987); [8] McGrogan et al., Biotechnology 6, 172 (1988); [9] Nick et al., Biochemistry, in press.

Neurotrophic Factors

Integrins and Cell Surface Receptors for Neurotrophic Factors

CR 003 FUNCTIONAL DOMAINS OF THE NGF RECEPTOR, Moses V. Chao, Hai Yan, Nila Patil, Enrique Escandon and Barbara Hempstead, Department of Cell Biology & Anatomy and Hematology/Oncology Division, Cornell University Medical College, New York, New York 10021.
The many trophic and differentiative effects of nerve growth factor (NGF) on sensory, sympathetic and cholinergic basal forebrain neurons are directly mediated by binding of NGF to a specific cell surface receptor. The NGF receptor is an integral membrane protein that is glycosylated and phosphorylated. The amino acid sequence and gene structure indicate that five discrete domains exist in the structure of the receptor. They include a single peptide sequence, a 160 amino acid region enrich in cysteine residues, a serine and threonine rich region, a single transmembrane region, and a cytoplasmic portion of 155 amino acids. We have introduced wild type and mutant forms of the NGF receptor into cultured cells, including fibroblasts and PC12 cells. Linker insertions and in-frame deletions have been introduced into the human receptor cDNA, and chimeric receptors have been constructed in order to define the functional domains of the NGF receptor molecule. In addition, we have detected neuronal-specific expression of the human NGF receptor gene in transgenic mice.

CR 004 RECEPTORS MEDIATING CELL ATTACHMENT AND NEURONAL PROCESS OUTGROWTH ON LAMININ D.O. Clegg, E.S. Choi, and J. Johnson, Neuroscience Research Institute, University of California, Santa Barbara, California 93106
Multiple classes of laminin receptors have been identified by two major experimental strategies: purification of proteins by laminin affinity chromatography and isolation of antibodies that block cell adhesion to laminin. Receptors include members of the integrin family of receptors, the 68 Kd receptor, crainin, and a 54 KD laminin binding protein from chick muscle. An important question in the field is to determine which receptors mediate cell adhesion and neurite outgrowth on laminin. For example, the $beta_1$ subunit of the integrin receptor family has been implicated in mediating neurite extension on laminin, but the identity of the alpha subunit(s) that combine with $beta_1$ to mediate process outgrowth on laminin have not been identified. At least four different alpha subunits can combine with $beta_1$ to form heterodimers that appear to interact with laminin : $alpha_1$, $alpha_2$, $alpha_3$, and $alpha_6$. The $alpha_3$-$beta_1$ heterodimer appears to be a promiscuous matrix receptor that can mediate cell attachment to laminin, collagen, and fibronectin.

We have analyzed the effects of function blocking anti-integrin monoclonal antibodies (Wayner and Carter, 1987) on process extension by the human neuroblastoma cell line SY-5Y, before and after differentiation induced by treatment with NGF and DNA synthesis inhibitors. When untreated SY5Y cells are plated on laminin, an anti-$alpha_3$ monoclonal P1B5 inhibited initial attachment, lamellapodia formation, and process formation. This antibody also induced rapid retraction of established processes. After treatment with NGF and DNA synthesis inhibitors, a differentiated subpopulation is selected that extends much longer processes. Process formation is inhibited much less by the anti-$alpha_3$ antibody. We conclude that the $alpha_3$-$beta_1$ integrin heterodimer can mediate process outgrowth on laminin and speculate that NGF treatment may alter neurite outgrowth behavior by altering the complement of integrin receptors expressed on the cell surface.

Wayner, E. A. and Carter, W.G. (1987) *J. Cell. Biol.*, **108**:1873-1884.

Neurotrophic Factors

CR 005 MEROSIN: A NERVE-AND MUSCLE-SPECIFIC LAMININ HOMOLOGUE, Eva Engvall, Ilmo Leivo, Karin Ehrig and Erkki Ruoslahti, La Jolla Cancer Research Foundation, 10901 North Torrey Pines Rd., La Jolla, CA 92037.

Basement membranes are morphologically similar in all tissues, but contain tissue-specific and differentiation-specific components that can be detected by immunohistochemistry. One of these tissue-restricted antigens is a protein that we have named merosin [Leivo and Engvall, PNAS 85, 1544 (1988)]. Merosin is present only in basement membranes of trophoblast and mature muscle and nerve. We have isolated and characterized the native merosin molecule from placenta. Electron microscopy after rotary shadowing shows that merosin has a cross-like structure similar to laminin. The molecular weight of merosin, estimated by SDS-PAGE, is about 700 kDa compared to 800 kDa for laminin. The purified merosin contains two 200 kDa light chains similar to the B1 and B2 light chains of laminin and these B chains are disulfide cross-linked to a 300 kDa merosin-specific heavy chain. The merosin heavy chain was cloned from a placental λgt11 cDNA library. Sequencing the COOH-terminal one-third of the merosin heavy chain showed that it is 40% homologous to the COOH-terminal portion of the heavy chain of laminin. Immunofluorescence on sections of various tissues with antibodies specific for the heavy chains of merosin or laminin shows that, in the adult, basement membranes contain either laminin or merosin but not both. The differential expression of laminin and merosin in basement membranes suggests different functions dependent on the heavy chains. The cell-binding and neurite-promoting activities of laminin are thought to reside in the COOH-terminal portion of the laminin heavy chain. We have found that merosin also interacts with cells and have begun to analyze the spectrum of cells and receptors recognizing merosin.

Neuronal Responses to Neurotrophic Factors-I

CR 006 DISSECTION OF THE NERVE GROWTH FACTOR MECHANISM OF ACTION WITH PURINE ANALOGS, Lloyd A. Greene, Cinzia Volonté, Alcmène Chalazonitis*, David Loeb, Hensin Tsao and Anna Batistatou, Dept. of Pathology and Center for Neurobiology and Behavior, Columbia University, College of Physicians and Surgeons, New York, NY 10032 and *Dept. of Neuroscience, AECOM, Bronx, NY 10461

Purine analogs have been used to dissect the NGF mechanism of action into distinguishable pathways. That is, these compounds inhibit some actions of NGF while sparing others. For instance, the analogs reversibly suppress neurite outgrowth from cultured PC12 cells, rat sensory neurons and rat and chick sympathetic neurons. In PC12 cultures, the analogs block the NGF-dependent inductions of c-*myc* mRNA and of ornithine decarboxylase (ODC) mRNA and enzymatic activity. On the other hand, the analogs do not block NGF-promoted neuronal survival or certain NGF-stimulated protein phosphorylation changes in PC12 cultures. A further distinction between NGF pathways is suggested by differences among the efficacies of various purine analogs for suppressing NGF actions. For example, the analog 2-aminopurine (2-AP) blocks NGF-promoted induction of mRNA's encoding the c-*fos* and c-*jun* proto-oncogenes, while the analog 6-thioguanine (6-TG) does not. Thus, the purine analogs appear to distinguish at least three different pathways in the NGF mechanism. One is blocked by many purines, including 2-AP and 6-TG. Another is sensitive to 2-AP, but not 6-TG, and a third is insensitive to purines. A possible mechanism by which the purine analogs may affect NGF actions is by interference with intracellular protein kinase activities. In particular, when tested in vitro, the analogs inhibited an NGF-activated protein kinase designated as protein kinase N (PKN). The latter appears to be a novel serine-threonine kinase that is rapidly activated by NGF. The relative potencies of the various analogs for in vitro inhibition of PKN were similar to those for inhibition of neurite outgrowth and ODC induction in intact cells. A number of criteria have failed to uncover other protein kinase activities that are blocked by 6-TG; 2-AP, in contrast, though far from non-specific as a kinase inhibitor, did not show absolute specificity for PKN. These and additional findings have raised the following hypotheses: 1) Protein kinases play a critical role in the NGF mechanism; 2) PKN activation is required for certain, but not all NGF actions; 3) 6-TG and other purine analogs suppress NGF actions by blocking PKN; 4) 2-AP affects a wider range of NGF actions than 6-TG because it is a less specific protein kinase inhibitor.

Neurotrophic Factors

CR 007 THE ROLE OF PHOSPHORYLATION IN THE ACTIONS OF NERVE GROWTH FACTOR,
Gordon Guroff, Section on Growth Factors, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892
Nerve growth factor is a peptide required for the survival and development of sympathetic and sensory neurons. It also acts on the cells of the adrenal medulla, certain tracts in the central nervous system, and on a number of tumors as well. Most recent studies on the mechanism by which nerve growth factor acts have been done with the model system PC12. This tumor, which arose in the adrenal gland of a New England Deaconess rat, differentiates under the influence of nerve growth factor from a chromaffin-like phenotype into a cell which markedly resembles a mature sympathetic neuron. In this model, and in normal target cells as well, the actions of nerve growth factor are accompanied by changes in the phosphorylation of a number of proteins in the cell. We have prepared and studied cell-free extracts from nerve growth factor-treated PC12 cells that reflect the prior treatment of the cells with nerve growth factor. That is, when cell-free extracts from treated cells are incubated with radioactive ATP they display the altered phosphorylation patterns seen in intact treated cells. Two such systems have been informative. In one, the phosphorylation of elongation factor 2 (EF-2) is decreased by nerve growth factor treatment. We have presented evidence indicating that the decrease in the activity of EF-2 kinase is caused by a phosphorylation of that kinase by kinase C. In another, the phosphorylation of the ribosomal protein S6 is increased in treated cells. We have presented evidence indicating that the increase in the activity of S6 kinase is due to a phosphorylation of that kinase by kinase A. Our working hypothesis, based on our data and on data from other laboratories, is that the combination of nerve growth factor with its receptor initiates a number of parallel phosphorylative cascades, leading to changes in the phosphorylation of a number of key proteins in the cell, including one or more in the nucleus. Further, we suggest that the actions of nerve growth factor are due to a summation of the changes in phosphorylation, and, accordingly, of function, of the increasing number of proteins whose phosphorylation is known to be altered by nerve growth factor. How the receptor is coupled to these kinase cascades is a subject of intense present research effort. The kinase inhibitor, K-252a, specific for the actions of nerve growth factor on PC12 cells, may provide the key tool by which this coupling can be understood.

CR 008 LIGAND-INDUCED TRANSIENT GENE EXPRESSION IN THE NERVOUS SYSTEM.
Harvey R. Herschman, Department of Biological Chemistry and Laboratory of Biomedical and Environmental Sciences, UCLA School of Medicine, Los Angeles, CA 90024.

Our laboratory has recently cloned a group of cDNAs for "primary response" genes that are rapidly and transiently induced in 3T3 cells by the tumor promoter tetradecanoyl phorbol actetate (TPA). We refer to these genes as TIS (TPA Induced Sequences) genes. The TIS1, 7, 8, 10, 11, 21 and 28 genes are all induced not only by TPA, but by epidermal growth factor and fibroblast growth factor, in 3T3 cells. The TIS28 cDNA is a partial c-fos clone. Because expression of the c-fos gene is known to be induced by nerve growth factor, as well as by a variety of other ligands, in PC12 cells, we examined the expression of the TIS genes in this cell line. We find that the TIS1, 7, 8, 11 and 21 genes can, like c-fos, be induced in PC12 cells by NGF. These genes can also be induced by TPA, EGF, FGF and carbachol in PC12 cells. In contrast, none of these ligands can induce the expression of TIS10 mRNA in PC12 cells; expression of this gene is "extinguished" in PC12 cells. The expression of TIS11B, an additional TIS gene cloned on the basis of sequence homology with the TIS11 gene, is also extinguished in PC12 cells. We suggest that part of the cellular specificity in biological responses is determined by the subsets of "immediate-early" genes that can be induced by extracellular ligands. We have also observed that the different TIS genes show differential induction by various ligands. Thus the TIS8 gene is equally well induced by NGF, FGF, and carbachol. In contrast, the TIS1 gene is strongly induced by carbachol, less well induced by NGF, and induced only poorly by FGF. We suggest that differential responsiveness of "immediate-early" genes, as a consequence of differential activation by the various second messenger pathways stimulated by alternative ligands, contributes to the distinguishable responses induced in target cells by alternative ligands, despite the existence of a relatively restricted number of potential second messenger pathways. Sequence data demonstrates that the TIS1 gene is the murine homologue of NGF1B, while TIS8 is the homologue of NGF1A. We have also examined the expression of the TIS genes in secondary rat glial cell cultures. We find that all the TIS genes can be induced in these cells by the mitogens EGF and FGF, and by the both cholinergic and adrenergic agonists. Pharmacologic experiments with appropriate antagonists and multiple ligand stimulation experiments suggest that induction of the various TIS genes occurs by the activation of multiple, independent second messenger pathways.

Neurotrophic Factors

CR 009 KINETIC AND MOLECULAR SPECIES ANALYSES OF AGONIST-INDUCED INCREASES IN DIGLYCERIDES, Daniel M. Raben[1], Melissa S. Pessin[1,2], Leela A. Rangan[1], Timothy M. Wright[3], Ralph A. Bradshaw[4], and Joseph G. Altin[4], [1]Departments of Physiology, [2]Neuroscience, and [3]Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, and [4]Department of Biological Chemistry, University of California, Irvine, College of Medicine, Irvine, CA 92717

It is now well established that the stimulation of cellular diglycerides plays an important role in the transduction of a number of agonist-stimulated events. Since diglycerides are present in unstimulated cells, activated events mediated by diglycerides must require: (a) a certain threshold level of diglyceride for activation; (b) appearance, disappearance, or change in the level of a certain molecular species upon stimulation, or possibly both (a) and (b). In addition, the subcellular compartment in which the diglyceride changes occur may also be important.

In view of the above, we have examined the kinetics of diglyceride stimulation in cultured IIC9 fibroblasts in response to α-thrombin, EGF and PDGF (1,2). In addition, we have analyzed the molecular species of these stimulated diglycerides, as well as those stimulated in PC12 cells by NGF, bFGF, and carbachol, by capillary gas chromatography (2). We also determined the molecular species profiles of the endogenous PI, PC, PE, and PS and compared them to the molecular species profiles of the stimulated diglycerides. We have also analyzed the release of radiolabeled water-soluble head groups in response to the various agonists. The data demonstrate: (a) kinetics of diglyceride generation are agonist-dependent; (b) PI is not the sole source of stimulated diglycerides; and (c) while PC is the likely source of non-PI-derived diglycerides, the molecular species profiles of stimulated diglycerides are not always identical to the profile of total cellular PC. The data support the hypothesis that some agonist stimulate the hydrolysis of a hormone-sensitive pool(s) of PC.

1. Wright, T.M., Rangan, L.A., Shin, H.S., and Raben, D.M. (1988) J. Biol. Chem. 263:9374.

2. Pessin, M.S., and Raben, D.M. (1989) J. Biol. Chem. 264:8729.

Neurotrophic Factors in Neurodegenerative Disease/Regeneration

CR 010 PROTEASE NEXINS: PROTEASE INHIBITORS IN BRAIN THAT REGULATE CULTURED NEURAL CELLS AND ARE ALTERED IN ALZHEIMER'S DISEASE. Dennis D. Cunningham, William E. Van Nostrand and Steven L. Wagner, Department of Microbiology and Molecular Genetics, University of California, Irvine, CA 92717

Protease nexin-1 (PN-1) and protease nexin-2 (PN-2) are protease inhibitors that are synthesized and secreted by a variety of cultured cells and occur in brain. They complex and inhibit certain serine proteases; some of the complexes are stable in SDS. The complexes bind back to the cells and are rapidly internalized and degraded. PN-1 is a specific thrombin inhibitor when bound to the cell surface. It is identical to the glial-derived neurite promoting factor or glial-derived nexin. It stimulates neurite outgrowth in cultured neuroblastoma cells; this activity depends on its ability to inhibit thrombin. Thrombin brings about neurite retraction in these cells. PN-1 and thrombin also recriprocally regulation the stellation of cultured astrocytes. The activity of PN-1 was reduced about 7-fold in Alzheimer's disease (AD) brain compared to age-matched control cases with similar postmortem times. The AD samples contained increased PN-1-containing complexes that co-migrated with PN-1-thrombin complexes. The PN-1 mRNA levels for PN-1 were about equal in the AD and control cases tested. Together, these results suggest that increased thrombin or a thrombin-like protease in AD brain leads to the formation of PN-1-thrombin complexes and a decline in free PN-1 (1). In normal human brain, much of the PN-1 occurs around blood vessels, suggesting a protective role against extravasated thrombin under conditions in which the blood brain barrier is compromised.

PN-2 recently was shown to be identical to the secreted form of the amyloid β-protein precursor. Other investigators have reported several effects of the precursor or its fragments on cultured fibroblasts and neurons. PN-2 reversibly inhibits chymotrypsin and plasmin and irreversibly inhibits trypsin and two closely related proteases, the EGF binding protein and the γ-subunit of NGF. An anti-PN-2 monoclonal antibody that binds an epitope in the first 25% of the amino terminal region of PN-2 binds to neuritic plaques in AD brain. The regulation of extracellular proteolysis by PN-2 and the deposition of at least parts of the molecule in senile plaques are consistent with previous studies which implicate altered proteolysis in the pathogenesis of AD (2).

1. Wagner, S. L. et al., Proc. Natl. Acad. Sci. U.S.A. **86**, 8284-8288 (1989)
2. Van Nostrand, W. E. et al. Nature **341**, 546-549 (1989)

Neurotrophic Factors

CR 011 NERVE GROWTH FACTOR EFFECTS ON INJURY AND REGENERATION OF ADULT CNS CHOLINERGIC NEURONS, Silvio Varon, Theo Hagg, H. Lee Vahlsing and Marston Manthorpe, Department of Biology, M-001, University California, San Diego, La Jolla CA 92093
Increasing evidence supports the hypothesis that neuronotrophic factors play crucial roles in the maintenance, function and repair capabilities of adult CNS neurons. Much information derives from the study of an adult rat septo-hippocampal model, where a complete fimbria-fornix transection separates cholinergic neurons from their innervation territory, the hippocampal formation (HF). Administration of Nerve Growth Factor (NGF), now known to act as a trophic factor for these neurons, both prevents and reverses damages incurred by the axotomized neurons. The new evidence to be reviewed here indicates that NGF also acts as a crucial controller of axonal regeneration from cholinergic septal neurons through a peripheral nerve graft placed as a bridge into the lesion cavity, and that it similarly controls the extent of penetration by the regenerating axons into their HF territory. Specifically : (1) sciatic nerve tissue deprived of its living cells (a proven source of NGF) no longer performs as a competent regeneration bridge, but recovers its competence when supplemented with exogenous NGF, and (2) the number of cholinergic fibers penetrating deeper regions of the HF increases several-fold upon intra-hippocampal administration of NGF. In more general terms, we speculate that the well-documented resistance to reinnervation of adult mammalian CNS tissue (contrasting with the low one of peripheral nerve) reflects mainly an insufficient local availability of endogenous neuronotrophic factors.

Neuronal Responses to Neurotrophic Factors-II

CR 012 NEURONAL EXPRESSION OF NGF AND FGF RECEPTORS DURING AVIAN DEVELOPMENT, Josef G. Heuer and Mark Bothwell, Department of Physiology & Biophysics, University of Washington School of Medicine, Seattle, WA 98195.
Acidic and basic fibroblast growth factors have neurotrophic activity similar to that of nerve growth factor for some neuronal cell types. We have employed 35S-labeled RNA probes to compare spatiotemporal patterns of expression of NGF and FGF receptors in developing chickens. The pattern of expression of the receptors for these two neurotrophic factors during development of the avian nervous system is quite different. In the peripheral nervous system, NGFR mRNA is expressed in relatively low levels in premigratory cells of neural crest and epibranchial placode and in immature cells of ganglia derived from these sources, and in higher levels in Schwann cells and fully differentiated neurons in sensory, sympathetic and parasympathetic ganglia. FGFR expression has been characterized thus far only in sensory ganglia. Immature ganglionic cells contain only very low levels of FGFR mRNA while in more mature ganglia, Schwann cells are negative while neurons are moderately positive. In the developing brain and spinal cord, NGFR mRNA is not expressed in periventricular neuroepithelial germinal cells, is expressed in moderate levels in postmitotic neuroblasts, is expressed transiently in high levels in many neuronal populations including various motor nuclei, and is present in lower levels or absent in corresponding locations in adult brain. In contrast, FGFR mRNA is present in neuroepithelial germinal cells, is absent in postmitotic neuroblasts, reappears in various neuronal populations including various motor nuclei late in development, and remains at relatively high levels in most of these cells in adult brain. Thus, while some neuronal cell types are transiently positive for both NGFR and FGFR mRNA, the differing timing of expression of receptors suggests that NGF and FGF have differing func-tional roles in brain development.

The FGF receptor cDNA clone used to generate probes for this study was provided by Dr.'s Pauline L. Lee, Daniel E. Johnson and Lewis T. Williams, whose contribution we wish to acknowledge.

Neurotrophic Factors

CR 013 TROPHIC ACTIONS OF IGF-I, bFGF, EGF AND TGF-α ON SEPTAL CHOLINERGIC AND MESENCEPHALIC DOPAMINERGIC NEURONS. F. Hefti, K.D. Beck, E.O. Junard, B. Knüsel, and G.M. Pasinetti. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

To investigate the response to trophic factors of cholinergic and dopaminergic neurons in the developing and adult brain, we analyzed the actions of several known growth factors on septal and mesencephalic neurons in culture and in animals with experimental lesions. IGF-I, IGF-II, and insulin stimulated survival of cholinergic neurons in culture and their expression of ChAT. In cultures of dopaminergic neurons these proteins elevated dopamine uptake. Actions of insulin, IGF-I, and IGF-II were not additive and the rank order of potency was IGF-I > IGF-II > insulin, suggesting that they are mediated by IGF-I receptors. Actions of insulin were additive to those of NGF and were not blocked by antibodies to NGF. In dopaminergic cultures, insulin elevated the number of cells stained with TH immunocytochemistry, increased TH mRNA levels per culture dish and per dopaminergic neurons when quantified with in situ hybridization, and stimulated TH activity. The action of insulin on cholinergic and dopaminergic cells was not dependent on the presence of glial cells. In contrast to these robust, direct trophic actions observed in a developing system, insulin, when given intraventricularly, was not able to counteract the lesion-induced degeneration of septal cholinergic or of nigral dopaminergic neurons in adult rats. bFGF, similar to NGF, elevated ChAT expression by cholinergic neurons and promoted their survival in vitro. These trophic actions were independent of those mediated by NGF. In dopaminergic cultures, bFGF elevated dopamine uptake and TH activity. Similar to NGF, bFGF, when given to adult rats with septo-hippocampal lesions prevented the degeneration of cholinergic cell bodies in the septum. In contrast, preliminary findings do not indicate any protective effect on dopaminergic neurons after lesions of the nigro-striatal pathway. EGF and TGF-α failed to influence cholinergic neurons in culture. In mesencephalic cultures, they elevated dopamine uptake, TH mRNA levels, TH activity and the number of cells stained with TH immunoreactivity. Actions of EGF and TGF-α were not additive and probably mediated by the same receptors.

The findings suggest that survival, growth and differentiation of septal cholinergic and mesencephalic dopaminergic neurons can be manipulated by various growth factors. NGF and bFGF seem to influence septal cholinergic neurons directly and rather selectively, and cholinergic neurons remain responsive to these factors in the adult brain. The neurotrophic actions of insulin and the IGFs appear to be more general and limited to developmental stages of cholinergic and dopaminergic neurons. Since cholinergic and dopaminergic neurons exhibit selective vulnerability in neurodegenerative diseases, these studies may provide a basis for the pharmacological use of neurotrophic factors.

Neurotrophic Factors

Neurotrophic and Growth Factors

CR 100 SENSITIVE ENZYME IMMUNOASSAY FOR RECOMBINANT HUMAN NGF, Gregory L. Bennett, Louis E. Burton, Wai-Pan Chan and Wai Lee T. Wong, Genentech Inc. S. San Francisco, CA 94080. A sensitive polyclonal antibody based Enzyme-Linked Immunosorbant Assay (ELISA) has been developed to quantitate human Nerve Growth Factor (hNGF). The polyclonal antibody (Ab), generated in a rabbit to E Coli derived rhNGF, was able to immunoprecipitate ^{125}I-labeled rhNGF and was positive in the Western Blot analysis. The Ab was purified by Protein A and used in the ELISA as the capture antibody as well as the conjugated antibody. The assay uses rhNGF expressed in mammalian cell as the standards and has a range of 0.05 - 6.25 µg/L with the lowest detection limit at 20 ng/L. Intra- and inter assay coefficients of variation were 6% and 10%, respectively. The specificity of the assay was tested with mouse NGF and shown to have little cross-reactivity with 2.5S(3%) and 7S (2%) forms, despite the high degree of sequence homology between human and mouse NGF. From the spike-recovery study (95-107%), the assay can accurately quantitate spiked rhNGF. The concentrations of rhNGF determined by the ELISA were compared with those obtained by the established neurite outgrowth PC12 bioassay and found to have good correlation. The assay has been used to measure rhNGF from various cell lines as well as NGF like immunoreactants in biological fluids.

CR 101 ANALYSIS OF THE NERVE GROWTH FACTOR (NGF) BINDING DOMAIN OF THE NGF RECEPTOR, Catherine M. Bitler, Andrew A. Welcher, Monte J. Radeke, and Eric M. Shooter, Department of Neurobiology, Stanford Medical School, Stanford, CA 93405
A structural analysis of the nerve growth factor receptor (NGFR) was undertaken to define the NGF binding domain of the low affinity NGFR. We have constructed a set of receptor cDNA clones with nested deletions at the 5' end, commencing after the signal peptide of the protein. We have also constructed two clones which encode 256 and 168 amino acids and have been designated the Nar and Bam clones, respectively. The Nar clone encodes the extracellular, transmembrane and intracellular portions of the NGFR protein, while the Bam clone encodes the extracellular region only. In order to expediate the study of NGF binding to the deleted receptors, a transient assay system was developed. The deleted receptor clones were subcloned into the transient expression vector, SRα . The SRα clones were transfected into monkey cos cells which resulted in a high level of expression of the NGFR deletion constructs. Two of the deletion constructs, (ΔLeu8-Cys58 and ΔLeu8-Val35) were able to bind ^{125}I-NGF, albeit at a lower level than WT. A third deletion construct, (ΔLeu8-Ile168), was unable to bind ^{125}I-NGF at all. The N-terminal deletion constructs were unable to bind MC192 as shown by FACS analysis. Interestingly, the Nar clone, as well as, the Bam clone (which is secreted into the media) were able to bind both ^{125}I-NGF and MC192. Thus, the site of binding for MC192 is distinct from that for NGF; alternatively, MC192 may require that the secondary structure of the receptor be retained (ie all disulfide bridges remain intact), while NGF does not.

CR 102 NON-RESPONDING MUTANT PC12 CELLS BIND NGF AFTER TRANSFECTION WITH THE NGF RECEPTOR cDNA, Laurel M. Bolin, Catherine A. Bitler, Andrew A. Welcher and Eric M. Shooter, Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305. The binding of nerve growth factor (NGF) to its receptor initiates a characteristic response in PC12 cells that includes neurite extension and changes in protein and messenger RNA expression. To study the mechanism of this signal transduction, mutant PC12 clones, prepared by chemical mutagenesis, were isolated that do not extend neurites in response to NGF. Of the non-responding clones isolated, six did not express low affinity NGF receptor (NGFR) message or protein as determined by Northern and Western blot analysis, respectively. Three of these clones have been transfected with the low affinity NGFR cDNA. The transfected clones have been analyzed for receptor protein by fluorescence activated cell sorting using MC192, a mouse monoclonal antibody specific for the rat low affinity NGFR. After several rounds of sorting and regrowth, clones that express high levels of the receptor protein were isolated. These transfected clones express levels of NGFR protein equivalent to normal PC12 cells, as determined by Western analysis. Crosslinking iodinated NGF to one of these transfected clones showed that it had regained its ability to bind NGF and to express the appropriate sized low affinity NGFR. The binding kinetics of the transfected and non-transfected mutant clones are currently being assayed. These mutant PC12 clones may allow us to determine whether or not transfection with the low affinity NGFR cDNA leads to reconstitution of high affinity NGF binding and the responses associated with high affinity binding.

Neurotrophic Factors

CR 103 EXPRESSION OF ACIDIC FIBROBLAST GROWTH FACTOR IN DEVELOPING RAT EYE,
Kuyas Bugra, David Hicks, Yves Courtois, INSERM Unit 118, Paris, France.
Several lines of evidence suggest that aFGF play a regulatory role in the development of the retina. To define timing of its expression in the eye we have used the reverse transcriptase coupled polymerase chain reaction with specific oligonucleotide probes from different exons. We have used total RNA from dissected eyes and retina at embryonic ages 12, 14, 18, and postnatal days 1, 5, 7, 9, 14 and the adults. The specificity of the amplified fragment was determined on the basis of its size (135bp), the presence of the predicted restriction sites, and its hybridization to aFGF probe but not to the bFGF probe. Quantifications were performed in the exponential phase of the amplification reaction where the extent of the amplification is proportional to the concentration of the specific transcript. The results were normalized with respect to results of co-reverse transcribed and co-amplified tobacco leaf nitrate reductase RNA in the same tubes . The specific expression of the aFGF is also compared to the expression of beta-tubulin and the photoreceptor specific opsin genes.

CR 104 ISOLATION OF THE CHICK NERVE GROWTH FACTOR GENE AND LOCALIZATION OF THE PROMOTER,
Alice Carrier and Charles Auffray, Institut d'Embryologie du CNRS et du Collège de France, 49bis, avenue de la Belle Gabrielle 94130 Nogent-sur-Marne, FRANCE.
We have isolated the chicken gene encoding β-nerve growth factor using a mouse cDNA probe. The DNA sequence of the 3' exon is relatively well conserved when compared to its mammalian counterpart, with up to 80% conserved nucleotides, and codes for pre-pro-NGF. In order to study regulation of NGF expression at the transcriptional level, we isolated the gene from a cosmid library, and after one chromosome walking step, have mapped 57 kb of DNA 5' to the coding exon to search for the promoter region. In birds, the most abundant source of NGF mRNA is heart and cerebral cortex where it represents only 1 in 10^6 transcripts, i.e. three orders of magnitude less than in mouse male submaxillary gland. This made isolation of full-length NGF cDNA impractical by standards techniques. We performed anchored-PCR with a 3' exon primer and amplified a 700 bp fragment that should contain the 5' end of NGF transcripts. In addition, hybridization of oligonucleotides derived from the rat and mouse NGF genes to our cosmid clones revealed two hybridizing bands located 53-55 kb upstream of the 3' exon. DNA sequence analysis of one of these bands revealed that it shares 18/21 nucleotides with the first non coding exon of mammals. Functional tests are in progress to demonstrate the promoter activity of this DNA segment, using CAT assays and DNA footprinting.

CR 105 IMMUNOHISTOCHEMICAL LOCALIZATION OF TRANSFORMING GROWTH FACTOR-BETAS 2 AND 3 IN THE NERVOUS SYSTEM OF THE MOUSE EMBRYO,
Kathleen C. Flanders, David S. Cissel, Anita B. Roberts, Michael B. Sporn, and Klaus Unsicker, Laboratory of Chemoprevention, NIH, Bethesda, MD 20892 and Dept. of Anatomy and Cell Biology, University of Marburg, Marburg, FRG.
Transforming growth factor-ßs (TGF-ßs) are multifunctional regulators of cell growth and differentiation which exist as 5 distinct, but highly homologous isoforms. We have developed antibodies specific to each TGF-ß to use for immunohistochemistry. In the mouse embryo, TGF-ßs 2 and 3 are expressed in both the peripheral and central nervous systems with similar localizations. At 12.5 d, immunoreactivity is very intense in the ventral marginal and mantle zones of the spinal cord and rhombencephalon, as opposed to the ventricular zone which has little staining. Both neurites and radial glial cells are stained. In older embryos (14-15 d), immunoreactivity is seen in the anlage of the cerebellum and the developing telencephalic cortex. In large motor neurons of the spinal cord and rhombencephalon, staining is localized within the perikaryon. At day 12.5, nerve fibers entering and leaving the spinal cord, rhombencephalon, dorsal root ganglia and ganglia of cranial nerves are strongly immunoreactive for TGF-ßs 2 and 3, while neuronal cell bodies within the peripheral ganglia are not yet stained; staining in the perikarya develops by day 15. Preliminary results suggest that TGF-ß3 is a neurotrophic agent for chick ciliary and dorsal root ganglia, while TGF-ß2 is not. The localizations of these TGF-ßs are also consistent with their involvement in control of neuronal migration, glial cell proliferation and differentiation, and neuronal-glial adhesion.

Neurotrophic Factors

CR 106 INTERLEUKIN-1 IMMUNOREACTIVITY IN PRIMARY RAT CORTICAL NEURONS AND ASTROCYTES,
Don C. Guiroy, Petra Friedrich and R. Yanagihara, Laboratory of Central Nervous System Studies, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland , 20892.

To determine the cellular localization of interleukin-1 (IL-1) in developing neurons and astrocytes, we stained cultures of primary dissociated rat cortical neurons and astrocyte by the peroxidase-antiperoxidase technique using a polyclonal antibody against murine IL-1. Immunoreactivity initially detected on day 7 in vitro in the cell body, axons, dendrites and growth cones of cortical neurons increased in intensity from day 14 to 28. IL-1 was also localized in the cell body, processes and growth cones of protoplasmic astrocytes, fibrous and multipolar astrocytes. On the other hand, immunostaining of neurons and astrocytes for phosphorylated neurofilament and glial fibrillary acidic protein, respectively, was detected at day 7 and remained unchanged during the 4-week observation period. Our data indicate that primary rat cortical neurons and astrocytes are immunoreactive to IL-1, suggesting that this cytokine plays an important role in the physiology, as well as, pathophysiology of cerebral function.

CR 107 EXPRESSION OF MURINE FIBROBLAST GROWTH FACTOR-5 IN THE NERVOUS SYSTEM,
Olivia Haub, Mitchell Goldfarb, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032.
Fibroblast growth factor-5 (FGF-5) is one of at least seven structurally related mitogens. By RNA filter blot analysis of adult mouse tissues, we detected FGF-5 mRNA in adult brain. Subsequent analysis of RNAs from parts of the central nervous system showed a broad distribution of FGF-5 expression with hippocampus, spinal cord, and cerebral cortex having somewhat higher mRNA levels than basal ganglia, cerebellum, midbrain and hindbrain. The hypothalamus was the only region in which we did not detect expression.
We further localized expression using in situ hybridization. We were able to detect FGF-5 RNA expression in the lateral reticular nucleus of the medulla, reticular nucleus of the thalamus, cerebral cortex (layers II through IV and layer VI), hippocampus (dentate gyrus and pyramidal cell layer in the CA3 region), piriform cortex, and olfactory tubercle. Detection in neuron-rich nuclei and cell layers suggests a neuronal basis for FGF-5 expression.
We are also examining embryonic FGF-5 expression. Preliminary results have detected expression in the developing peripheral nervous system as well as in portions of sclerotomes and other embryonic mesenchyme.

CR 108 COMPARISON OF THE TRANSCRIPTS FOR HUMAN NGF FROM A cDNA GENE AND A SYNTHETIC GENE WITH ALTERED CODON USAGE,
Mats Lake, Helena Wiman, Anne Kinhult, Kerstin Schenström, Annelie Sjögren, Christina Henrichson, Katarina Krook, Torsten Sejlitz and Lennart Hansson, KabiGen AB, Strandbergsgatan 49, S-112 87 Stockholm, Sweden
Nerve growth factor (NGF) is a 118 amino acid basic polypeptide required in its dimeric form for differentiation and proliferation of neurons.
The cDNA coding for the human β-NGF prepro-polypeptide was cloned into various BPV-1 based expression vectors containing different mRNA processing signals. Substitution of the original signal sequence was analysed. A synthetic NGF encoding sequence with altered codon usage was also evaluated. Stable cell lines producing human β-NGF were obtained after transfection of mouse C 127 cells. The mRNA stability in the different transformed cells were compared and expression of extracellular dimers were determined by an elisa and correlated to the biological activity.
The product was recovered from the medium and was purified to homogeneity by chromatographic tehniques. The protein was analysed by SDS-PAGE with following western blot and the human β-NGF migrated as a monomer of 13kD.

CR 109 TWO ANION EXCHANGE CHROMATOGRAPHIES ENCOMPASSING AN ENZYMATIC DIGESTION STEP ALLOW DETERMINATION OF NEURITE PROMOTING ACTIVITIES OF DIFFERENT LAMININ/PROTEOGLYCAN COMPLEXES AND THEIR DEGRADATION PRODUCTS. Peter H. Matthiessen, Corinne Schmalenbach and Hans Werner Müller, Molecular Neurobiology Laboratory, Department of Neurology, University of Düsseldorf, F.R.G.
Conditioned media (CM) of different adherent primary cell cultures or cell lines contain neurite promoting activities (NPA) for various peripheral and central neurons which have been recently characterized in some cases as laminin/proteoglycan complexes. We have separated concentrated meningeal cell CM by anion exchange chromatography on Mono Q and have measured the NPA of the PLL-adherent part of the fractions using a quantitative bioassay with embryonic (E18) neurons from rat hippocampus. In the main strong peak of NPA being eluted at 950-1400 mM sodium chloride we could detect by ELISA overlapping immunoreactivites for a heparan sulfate proteoglycan core protein (B3) and laminin (LN) separated from those specific for chondroitin sulfate proteoglycan (ChSPG) and LN. The presence of separate proteoglycan complexles consisting of proteoglycans bearing one type of glycosaminoglycan could be supported by specific degradation of the B3/LN complex by heparitinase and the ChSPG/LN complex by chondroitinase ABC. Mono Q-fractions of the second column run after enzymatic digestion revealed a total decrease in NPA in the previously active fractions, whereas the degradation products, B3 core protein and free LN only showed minor NPA. Supported by the DFG (Mu 630/3-2).

CR 110 MOLECULAR CLONING OF THE RABBIT CNTF GENE. Drzislav Mismer, Jack D. Lile and Frank Collins. Synergen, Inc., 1885 33rd Street, Boulder CO 80303.
We have cloned the rabbit ciliary neurotrophic factor (CNTF) gene by using PCR amplification. In our reactions two sets of degenerate oligonucleotides plus 0.5 µg of rabbit genomic DNA were used to amplify portions of the rabbit gene. The subclones of amplified products were then used to obtain CNTF clones from a rabbit genomic library as well as a cDNA library prepared from poly(A)$^{+}$-RNA isolated from sciatic nerve. The primary structure of rabbit CNTF was determined by dideoxy DNA sequencing of a single cDNA clone and confirmed by the sequencing of the genomic clones. The inferred rabbit protein is 199 amino acids long and has a calculated pI of 5.79, which agrees well with the molecular weight and pI observed for purified CNTF. The rabbit cDNA was transiently expressed in COS-7 cells and produced a protein with immunological, physical-chemical, and biological properties of CNTF (Science 246: 1023-1025). The mRNA encoding rabbit CNTF is 4.3 kb in length. The coding sequences are interrupted by a single intervening sequence, approximately 1.3 kb in length, located after the Tyr^{38} residue.

CR 111 HIGH LEVEL EXPRESSION OF RECOMBINANT hNGF IN MAMMALIAN CELLS. Sharon Ogden, Suzanne Bolten, Kumnan Paik, Pamela Breen and Pamela Manning. Monsanto Co., Chesterfield, MO 63198.
Nerve growth factor (NGF) is a 118 amino acid protein which serves an important role in the development of the sympathetic and sensory nervous systems. In addition to its function as a peripheral neurotrophic factor, NGF has also been shown to have physiological effects on cholinergic neurons in the brain. Although there exists a convenient preparative source of mouse NGF (male mouse submaxillary gland, MSG), no comparable source of human NGF (hNGF) exists. In order to obtain hNGF, we expressed the hNGF gene in mammalian cells. We obtained high level expression (>10 µg/10^6 cells/day) of hNGF in several different mammalian expression systems. The purified protein has a specific activity at least equivalent to MSG-derived mNGF and behaves comparably in a number of bioassays. 2-D Western blot and sequence analysis indicates that the recombinant hNGF is properly processed in all of the cell lines tested.

Neurotrophic Factors

CR 112 CHARACTERIZATION OF A PUTATIVE FIBROBLAST GROWTH FACTOR RECEPTOR FOR ACIDIC AND BASIC FIBROBLAST GROWTH FACTORS, Bradley B. Olwin, Laura W. Burrus, Arthur J. Kudla, Bruce A. Lueddecke, Daniel J. Shaw and Michael E. Zuber. Department of Biochemistry, University of Wisconsin, Madison, WI 53706.

Basic and acidic fibroblast growth factors (bFGF, aFGF) are potent neurotrophic agents for both peripheral and CNS neurons. We have recently isolated a putative FGF receptor from embryonic chick that binds both aFGF and bFGF (Burrus and Olwin, JBC, 264:18647). Monoclonal antibodies were generated to the FGF receptor present in this partially purified preparation. Three monoclonal antibodies recognize distinct epitopes of a 150 kDa glycoprotein in Western blots of crude chick membranes. Immunoadsorbed FGF receptor retains high affinity binding to ^{125}I-bFGF that is inhibited by heparin, and is displaced by both aFGF and bFGF. Western blot analysis of immunoreactive 150 kDa FGF receptor during chick embryogenesis suggests FGF receptor is present in most developing tissues, with the highest levels in brain and eye. Immunolocalization of the 150 kDa FGF receptor in frozen sections demonstrates a majority of receptor is present in the developing CNS. Together, these data suggest a role for the 150 kDa FGF receptor in the developing CNS of the chick. In agreement with these results, analysis of ^{125}I-aFGF binding during chick development reveals a constant level of ^{125}I-aFGF binding from day 2 to day 6 of incubation, followed by a rapid decline to a constant low level of ^{125}I-aFGF binding in the brain, eye, and liver. Loss of ^{125}I-aFGF binding and immunoreactive 150 kDa FGF receptor occurs between day 7 and day 19 of embryogenesis in both skeletal muscle and heart tissue. Preliminary sequencing of cDNA clones encoding the 150 kDa putative FGF receptor indicate that this receptor is distinct from a protein tyrosine kinase cDNA isolated from chick embryos (Lee *et al.*, Science, 245:57; Pasquale *et al.*, PNAS, 86:5449) and its human homolog, flg, that binds both aFGF and bFGF (Ruta *et al.*, PNAS, 86: 8722).

CR 113 EXPRESSION AND PURIFICATION OF RECOMBINANT RAT CILIARY NEUROTROPHIC FACTOR (RCNTF), Nikos Panayotatos, Piotr Masiakowski, Czeslaw Radziejewski, Vivien Wong and Haoxing Liu, Regeneron Pharmaceuticals, Inc. 777 Old Saw Mill River Road, Tarrytown, NY 10591. Ciliary neurotrophic factor (CNTF) is a protein involved in the survival, proliferation and differentiation of embryonic neurons and the differentiation of type-2 astrocytes. Recently, the rat CNTF gene was cloned and expressed in HeLa cells (Stockli, *et al. Nature*, in press). A DNA fragment coding for the RCNTF gene was obtained from the cDNA clone by PCR technology and introduced in *E. coli* expression vectors. In the optimal system, the recombinant protein amounted to 60-70% of total cellular protein and it was present mostly in inclusion bodies. Extraction by guanidinium hydrochloride followed by a single column chromatography step produced high yields of RCNTF. The recombinant protein was better than 99% pure, was pyrogen-free and was biologically fully active in supporting neurite outgrowth from embryonic chick ciliary neurons in culture at the nanogram level.

CR 114 NEURONAL CHARACTERISTICS OF PC12 CELLS INDUCED BY KIRSTEN-ras ONCOGENE: COMPARISON TO NERVE GROWTH FACTOR. D.L. Simpson°, J. Tao-Cheng°, J.P. Bressler#, O. Okuda, L. Chang° and M.W. Brightman°. Lab of Neurobiology°, NINDS, NIH, Bethesda, MD 20892, Kennedy Institute#, Baltimore, MD 21205, and Juntendo University, Tokyo.

An intriguing relationship exists in PC12 cells between genes thought to regulate normal cell division and differentiation and oncogenes capable of transforming cells. That PC12 cells might serve as donors for implantation to brain regions deficient in certain neurotransmitters, led us to investigate whether K-ras differentiated PC12 cells exhibit the same neuronal morphology and neurochemical alterations as those differentiated by NGF. K-ras infected PC12 cells ceased dividing and developed neurites resembling those of NGF-treated cells or neurons in primary culture. Two characteristic types of secretory vesicles, large dense core granules (100 nM) and small clear vesicles (45 nM) abound. Compared to control cells NGF-treated cells and K-ras infected cells had elevated levels of choline acetyltransferase, acetylcholinesterase and tyrosine hydroxylase. Catecholamine analysis by HPLC and electrochemical detection suggested both dopamine and norepinephrine levels were reduced, but appreciable, in K-ras and NGF-treated cells compared to non-treated controls. Thus, K-ras infected cells become non-tumorogenic, maintain their neuronal phenotype and can synthesize significant levels of acetylcholine and catecholamines for extended periods of time, *in vitro*, making them prime candidates for neural implantation.

CR 115 ROLE OF A NEUROTROPHIC PROTEIN, S100β, IN GLIAL AND NEURONAL CELL FUNCTION.
Linda J. Van Eldik, Steven W. Barger, and Richard H. Selinfreund, Depts of Pharmacology and Cell Biology, Vanderbilt University, Nashville, TN 37232.
A disulfide form of S100β, a protein abundant in glial cells, has been found to have neurotrophic activity on CNS neurons in vitro. This observation, coupled with the presence of extracellular S100 in brain and glial cell cultures, raises the possibility that S100 might be released from glial cells and act in a paracrine fashion on neuronal cells. In order to approach the longer-term question of the role of S100 in CNS development and maintenance, we produced S100β protein (termed VUSB-1) by recombinant DNA technology (Van Eldik et al, 1988, J Biol Chem 263:7830), and showed that VUSB-1 preparations have selective, specific neurotrophic activity on embryonic chick cortical neurons. VUSB-1 enhances cell maintenance and stimulates neurite outgrowth. We also showed (Winningham-Major et al, 1989, J Cell Biol 109:3063) by site-directed mutagenesis/protein engineering approaches that both of the two cysteine residues of S100β are important for activity, but that the relative positions of the cysteines can be altered to some extent without loss of activity. Our more recent studies are aimed at elucidating the functional consequences of aberrant expression of the S100β gene in glial cells. The data to date suggest that inhibition of S100β in rat C6 glioma cells by inducing an antisense S100β gene or by using antisense S100β oligonucleotides results in altered cellular morphology and growth rate. Altogether, our studies are providing insight into potential roles for S100β in growth and differentiation processes in the CNS, and a knowledge base for longer-term drug development targeted at CNS maintenance and repair. (Supported in part by funds from Muscular Dystrophy Association, Cystic Fibrosis Foundation, and American Paralysis Association).

CR 116 INFLUENCE OF FGF ON THE PROLIFERATION AND DIFFERENTIATION OF MOUSE NEURAL PRECURSOR CELLS *IN VITRO*. Peter J. Wookey, Mark Murphy, John Drago and Perry F. Bartlett. Walter and Eliza Hall Institute, Parkville, Victoria 3050, Australia. The mammalian central nervous system develops from the neuroepithelial cells of the neural tube, and two major cell types of the CNS, neurons and glia, have previously been shown to originate from neuroepithelial cells *in vitro*. We have tested the possibility that growth factors, normally present in tissues of the developing and mature CNS, may be involved in the process of proliferation and differentiation of neural precursor cells. Of the factors tested using an *in vitro* culture system of neural precursor cells from neuroepithelium of embryonic day 10 mice, only FGF stimulates proliferation and differentiation. At least 50% of the neuroepithelial cells divide in the presence of FGF whereas in its absence all of the cells die within six days. At higher concentrations of FGF, cells change from being nonadherent round cells in tight clusters into a more flattened morphology and adhere to the substratum. The morphological changes that eventuate, are accompanied by expression of both neurofilament and GFAP, markers for neurons and glia respectively. In addition, an immortalised neuroepithelial cell line, itself independent of FGF for proliferation, expresses both markers in response to FGF. Data are presented which support the idea that FGF is involved in the process of proliferation and differentiation of neural precursor cells.

Neurotrophic Factors

Neuronal Responses

CR 201 GLUTAMIC ACID DECARBOXYLASE-GAD AND PREPROENKEPHALIN-PPE MESSENGERS ARE DIFFERENTIALLY REGULATED BY SELECTIVE D1 OR D2 AGENTS IN THE RAT STRIATUM, Caboche Jocelyne, 'Vernier Philippe, Rogard Monique, 'Julien Jean François, 'Mallet Jacques and Besson Marie-Jo, Neurochimie-Anatomie, IDN, Université Paris 6, 9 quai St-Bernard, 75005 Paris and 'Neurobiologie Cellulaire et Moléculaire, CNRS, 91198 Gif/Yvette, FRANCE.

The interruption of Dopaminergic-DA innervation produces increased levels of GAD- and PPE-mRNAs in the rat striatum (Tang et al., 1984; Vernier et al., 1988) suggesting a tonic inhibitory control of DA. Since DA can act at least through two receptor subtypes (D1 receptors activating adenylate cyclase, and D2 receptors inhibiting this enzyme but also phospholipase C), we investigated the precise role of each DA receptor subtype, on the regulation of GAD and PPE gene expression in the dorsal striatum of the rat. Specific mRNAs were identified by the northern-blot technic using ^{32}P-cDNA probes and quantified by densitometry. The chronic blockade of D2 receptors with (D-L)Sulpiride, a selective D2 antagonist, increased similarly GAD-and PPE-mRNA levels (+80%) and this effect was mimicked by the chronic administration of haloperidol. On the contrary, the chronic blockade of D1 receptors with the D1 antagonist, SCH 23390 compound, produced a significant decrease of both messengers(-30%). The coadministration of SCH 23390 and RU 24926 (a D2 agonist) potentiated the decrease of PPE-mRNA (-60%) and GAD-mRNA (-40%) levels. Our results suggest that DA acting through D2 receptors exerts a tonic inhibitory control on the expression of GAD and PPE messengers. This regulation might be related to a cAMP-dependant and/or a phorbol ester-dependant mechanism.

CR 202 THE ONTOGENY OF TRANSFERRIN RECEPTORS IN NEURONS OF THE DEVELOPING CHICK RETINA, Sa Sun Cho and Arnold G. Hyndman, Department of Biological Sciences, Rutgers University, Piscataway, NJ 07060. Transferrin is a growth factor likely to play a significant role in CNS development. In the developing chick retina, transferrin is uniquely located in the synaptic and nerve fiber regions and in the outer segments of photoreceptors. For the first time, the appearance of the transferrin receptor (TfR) in the chick retina is examined using a specific anti-body for the chick TfR. TfR immunoreactivity is first detectable throughout the retina at E (embryonic day) 4. With further development, TfR immunoreactivity becomes concentrated in the inner nuclear layer, the ganglion cell layer and outer segments of photoreceptor cells. In contrast to transferrin, TfR appears to be located primarily on soma. Immunoreactivity in the ganglion cell layer discretely defines a population of neurons. The number of TfR cells in the ganglion cell layer is less than 1,000 from E4 to E9. From E10 to E15 this number increases to about 40,000 and then falls to 30,000 by hatching. Within the ganglion cell layer TfR cells first appear along the central portion of the optic streak and later appear in more peripheral regions of the retina. Two distinct types of TfR cells are observed. Neurons with an ovoid morphology and unipolar or bipolar processes are noted between E6 and E15 and are located primarily near the optic streak. TfR multipolar neurons appear at E15 and are uniformly distributed throughout the retina, expect for around the optic streak and at the extreme periphery.

CR 203 RESPONSE OF NON-CONTACTING NEURONS FROM MEDIAL BASAL FORE-BRAIN CULTURED IN A THREE-DIMENSIONAL MATRIX TO NERVE GROWTH FACTOR, P.W. Coates, E. Dunn and M.S. Walker, Department of Cell Biology & Anatomy, Texas Tech University HSC School of Medicine, Lubbock, TX 79430.

The response of cholinergic neurons to nerve growth factor (NGF) may be affected by direct contact with other cells. It is unknown whether neurons from cholinergic regions such as medial basal forebrain (MBF) respond *intrinsically* to NGF without cell-contact mediated mechanisms, because complex and multiple connections ordinarily form between neurons and glia in vivo and in standard culture models. However, when neurons from MBF are grown in three-dimensional culture at low cell density the neurons remain single and physically separate from each other, yet rapidly express characteristic neuronal properties. Survival and growth/differentiation of such neurons cultured with and without NGF in this system were measured and analyzed quantitatively and qualitatively. Results suggest that neurons from MBF are *inherently* capable of responding to NGF independent of synaptic or glial interaction. This system should be useful for examining molecular mechanisms regulated by NGF alone, without influences provided by direct cell-cell communication. (Supported by NS20802, HD22806, SITRF and ADRDA).

Neurotrophic Factors

CR 204 AGRIN: A SYNAPTIC ORGANIZING MOLECULE CLOSELY RELATED TO NON_SYNAPTIC BASAL LAMINA PROTEINS. Earl W. Godfrey. Dept. of Anatomy & Cellular Biol., Medical College of Wisconsin, Milwaukee WI 53226. Agrin is a synapse-organizing protein that is found in motor neurons and concentrated in the synaptic basal lamina of the neuromuscular junction; agrin is also associated with the basal laminae of many tissues. Agrin purified from ECM-enriched fractions of several Torpedo tissues, including skeletal muscle, heart and intestine, is similar biochemically to agrin from the nervous system and electric organ, and aggregates acetylcholine receptors (AChRs) on cultured skeletal myotubes regardless of tissue source (Godfrey et al., J. Cell Biol. 106: 1263-1272, 1988).

Here I report that, in the chicken, agrin-like proteins were associated with most basement membranes as well as the cytoplasm of spinal cord motor neurons. Affinity-purified agrin preparations from non-neural tissues such as kidney and muscle often induced a smaller number of AChR aggregates on myotubes than did preparations from embryonic brain or spinal cord. However, a few preparations from adult chicken kidney induced as many clusters as agrin from embryonic brain or spinal cord. This suggests that non-neural tissues in the chicken contain inhibitor(s) of agrin activity that co-purify with the agrin-like proteins, or that non-neural agrin is more susceptible to inactivation during preparation. The major agrin-like proteins from neural and non-neural tissues were biochemically and immunologically indistinguishable. Antisera against agrin-like proteins from kidney blocked and precipitated agrin activity from brain, spinal cord and Torpedo electric organ. Thus, agrin-like proteins of non-neural tissues in chicken are closely related to agrin from the nervous system.

Supported by NIH grants HD20743 and NS27218.

CR 205 *RAS* PROTO-ONCOGENE METABOLISM AND POSTTRANSLATIONAL MODIFICATION IN THE PC12 NEURONAL CELL LINE: RELEVANCE TO QUESTION OF *RAS* INVOLVEMENT IN NGF SIGNAL TRANSDUCTION, Steven H. Green, Meng-Sheng Qiu & Andrew F. Pitts, Department of Biology, University of Iowa, Iowa City, IA 52242

In order to investigate the mechanism by which NGF or transforming *ras* induce neuronal differentiation in PC12 cells, we have studied the expression, metabolism and posttranslational modification of *ras* in these cells. With regard to posttranslational modifications, we have focussed on the polyisoprenylation of *ras*. We have determined the kinetics of *ras* turnover as well as turnover of the polyisoprene tail by immunoprecipitation of ^{35}S-methionine- and ^{3}H-mevalonate-labeled *ras* in pulse-chase experiments. These kinetics have been determined for the endogenous PC12 cell *ras* as well as for a transforming *ras* expressed from a dexamethasone-inducible (MMTV) promoter in UR61, a stably transfected PC12 cell line. Polyisoprenylation of *ras* in PC12 cells can be inhibited by compactin or lovastatin (which inhibit synthesis of mevalonate, the precursor to the polyisoprene moiety that is covalently linked to *ras*). Compactin treatment of PC12 cells inhibits proteolytic cleavage of the CAAX tail, translocation of *ras* to the membrane, and the responses of UR61 cells to expression of transforming *ras*. Thus, polyisoprenylation of *ras* appears to be necessary for function of *ras* in induction of neuronal differentiation in PC12 cells. Function and/or membrane localization of *ras* appears also to be involved in the regulation of expression of *ras* genes: treatment of PC12 cells with compactin results in a marked increase in *ras* expression. However, compactin does not appear to inhibit PC12 responses to NGF: NGF-dependent cell survival, NGF-induced phosphorylation of tyrosine hydroxylase and NGF-dependent induction of certain transcripts, determined by Northern blot analysis. Compactin does inhibit NGF-induced cell surface ruffling but this effect is nonspecific: EGF-induced ruffling is similarly inhibited by compactin. Thus *ras* function may not be necessary for responses to NGF. These observations are not consistent with a hypothesis that *ras* is involved in the NGF signal transduction pathway.

CR 206 THE HUMAN NGF RECEPTOR: DELETION OF CYTOPLASMIC SEQUENCES OF THE RECEPTOR LEADS TO LOSS OF HIGH AFFINITY LIGAND BINDING, Barbara L. Hempstead, Nila Patil, Bonnie Theil and Moses V. Chao, Division of Hematology and Department of Cell Biology and Anatomy, Cornell University Medical College, New York, New York, 10021. The nerve growth factor (NGF) receptor is a glycosylated transmembrane protein present on the cell surface as both high and low affinity forms, but biological responsiveness requires interactions of NGF with the high affinity site. We have tested the effects of mutations in the intracellular domain of the receptor upon its cell surface expression and equilibrium binding of ^{125}I-NGF. Although mutant receptors lacking the entire cytoplasmic domain are processed and expressed at the cell surface, and are capable of binding to NGF, the absence of cytoplasmic sequences leads to a loss of high affinity binding and to a lack of an appropriate crosslinking pattern as assessed by HSAB photoaffinity crosslinking. These results, taken together with the highly conserved nature of these cytoplasmic sequences, implies that the interaction of the receptor with an accessory molecule is necessary to form a high affinity receptor.

Neurotrophic Factors

CR 207 NERVE GROWTH FACTOR DEPENDENCE OF CULTURED SYMPATHETIC NEURONS IS DETERMINED BY CYTOPLASMIC FREE CALCIUM, Tatsuro Koike and Shuitsu Tanaka, Department of Natural Science, Saga Medical School, Nabeshima, Saga 84001, Japan.
Sympathetic neurons depend on nerve growth factor (NGF) for their survival both in vivo and in vitro; these cells die upon acute deprivation of NGF. We have previously shown (PNAS 86:6421-6425, 1989) that high K^+ medium prevents NGF deprivation-induced death of superior cervical ganglion (SCG) cells that have been cultured for 1 week in the presence of NGF. Experiments using Ca^{2+} channel antagonists and agonists demonstrate that Ca^{2+} influx through dihydropyridine-sensitive Ca^{2+} channels plays a major role in the survival promoting effect of high K^+. Indeed, free Ca^{2+} measurements using fura-2 fluorescence imaging reveal that the concentration of cytoplasmic free Ca^{2+} , $(Ca^{2+})i$, of the cells maintained in high K^+ (35mM) medium is elevated and sustained to 220-240nM in comparison to that under normal conditions (ca 106nM). The rise in $(Ca^{2+})i$ under depolarizing conditions was abolished upon replacement of the high K^+ by normal medium, and the neurons eventually died in the absence of NGF. Based on these results we propose a Ca^{2+} set-point hypothesis for the degree of NGF dependence of sympathetic neurons in vitro. We have tested this hypothesis by measuring basal $(Ca^{2+})i$ of SCG neurons as a function of incubation time in tissue culture. The basal $(Ca^{2+})i$ remained relatively low (ca 80nM) at day 3, increased with incubation time, and reached a plateau level of 220-250nM by week 3 when these neurons become independent of NGF for their survival. These results are in good agreement with the Ca^{2+} set-point hypothesis on the development of NGF independence of sympathetic neurons in culture.

CR 208 SYNAPTIC REMODELLING IN STRIATUM: MOLECULAR AND MORPHOLOGICAL CORRELATES, Giulio M. Pasinetti, Heng-Wei Cheng, Caleb E. Finch and Thomas H. McNeill, Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089-0191.
The molecular mechanisms which correlate with the formation of new synapses in the striatum (ST) following cortical deafferentation were examined. We used northern blot analysis to assay mRNA prevalence for the growth cone associated protein, GAP-43; the astrocytic specific glial fibrillary acidic protein, GFAP; the developmentally regulated intermediate filament, vimentin and the sulfated glycoprotein, SGP-2. Changes in mRNA prevalence were correlated with variations in the immunocytochemical staining patterns for GFAP and tyrosine hydroxylase (TH) in both the ipsilateral and contralateral ST. We found that mRNA prevalence for GFAP and SGP-2 in the ipsilateral ST was increased at 3 days postlesion and reached a maximum (8-10 fold) at 10 days postlesion. In contrast, the increase in mRNA prevalence for vimentin (50 fold) was greatest at 3 days postlesion and remained elevated, but to a lesser degree, at 10 and 27 days postlesion. GAP-43 mRNA prevalence in the ipsilateral ST was not changed at any time point. In the contralateral cortex, the mRNA prevalence for GFAP, SGP-2 and vimentin were all increased by 3 days postlesion (3 fold) and declined to control values by 27 days postlesion. GAP-43 mRNA prevalence in the contralateral cortex was variable and increased only slightly (70%) at 10 and 27 days postlesion. The increase in GFAP and SGP-2 mRNA prevalence in the ipsilateral ST was correlated with an increase in the density of immunoreactive fibers for both GFAP and TH in the dorsolateral part of the ST. In situ hybridization studies are ongoing and will be used to clarify the cellular relationship between the molecular and morphological changes described in response to cortical deafferentation of the ST.

CR 209 CELL CYCLE-SPECIFIC ACTION OF NERVE GROWTH FACTOR IN PC12 CELLS: DIFFERENTIATION WITHOUT PROLIFERATION, **Brian B. Rudkin**, Philip Lazarovici, Ben-Zion Levi[1], Yuya Abe[2], Ko Fujita and Gordon Guroff, **Section on Growth Factors** and [1]Laboratory of Developmental and Molecular Immunity, Section on Molecular Genetics of Immunity, **National Institute of Child Health and Human Development**, [2]Laboratory of Biological Chemistry, Division of Cancer Treatment, Developmental Therapeutics Program, National Cancer Institute, **National Institutes of Health, Bethesda, MD 20892**. PC12 cells were manipulated in such a way as to permit the study of differentiation-specific responses independently from proliferative responses. Cells were starved for serum then exposed to nerve growth factor (NGF) or serum. Following addition of serum, cells incorporated thymidine in a synchronous manner. Subsequent to the wave of DNA synthesis, the cell number increased approximately two-fold. Addition of NGF to serum-starved cultures had no measureable effect on either parameter. Neurite outgrowth was more rapid and extensive, and appearance of Na^+ channels, measured as saxitoxin binding sites, more rapid than when NGF was added to exponentially-growing cells. Epidermal growth factor receptors were heterologously downregulated by NGF with similar kinetics under both conditions. Induction of the proto-oncogene c-fos by NGF was also greater in the serum-starved cells than in exponentially-growing cultures. These results indicated that serum starvation resulted in synchronisation of the cultures and that NGF action may be cell cycle-specific. Analysis of the cellular response to NGF at different times during the cell cycle showed that c-fos was induced in the G_1 phase but not in S or G_2. Fluorescence-activated cell sorter analysis demonstrated that addition of NGF to exponentially-growing cells, resulted in their accumulation in a G_1-like state. With regard to the study of the mechanism of NGF action, these results illustrate that measurements of NGF effects on specific components in the signal transduction pathway may be confounded by the use of exponentially-growing cultures.

CR 210 **THE DEVELOPMENT OF THE ENTERIC NERVOUS SYSTEM IN THE ABSENCE OF VAGAL NEURAL CREST CELLS**, D. Tibboel, J.M. van Dongen, J.H.C. Meijers, A.W.M. van der Kamp and J.C. Molenaar. Depts of Cell Biology and Pediatric Surgery. Erasmus University Medical School, P.O.Box 1738, 3000 DR Rotterdam, The Netherlands.

It is generally agreed that the autonomous nervous system of the digestive tract derives from the vagal neural crest (NC), but there is controversy about a sacral NC source. We studied the possible contribution of the sacral NC to enteric neurons in the avian hindgut. Craniocaudal migration of vagal NC cells was prevented by transecting the bowel in four-day-old (E4) chicken embryos. Subsequently, the bowel developed in situ allowing the colonization by sacral NC cells. The embryos were fixed at various stages of development (E6-E16) and the intestines were prepared for LM and EM studies using HNK-1 and antineurofilament antibodies. No enteric neurons were present in the distal bowel. We observed HNK-1 positive unmyelinated axon bundles at the site of the myenteric plexus. The axon bundles completely filled the space of the myenteric plexus; occasionally we observed single cells in the myenteric region lacking the neuronal phenotype.

We conclude that 1) sacral NC cells do not give rise to enteric neurons in the hindgut, 2) axon bundles find a target in aneuronal bowel at the site where myenteric ganglia will normally develop. Extrinsic nerve fibers develop normally in the gut without nerve cells present. The model provides new insights into the pathogenesis of congenital innervation anomalies of the gut such as Hirschsprung's disease.

CR 211 EXOGENOUS NGF STIMULATES ACETYLCHOLINE RELEASE IN AGED FISHER MALE RATS. Larry R. Williams and R. Jane Rylett. CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001 and Dept. Physiology, Univ. of Western Ontario, London, Canada. Previous experiments found that chronic ICV administration of exogenous NGF stimulates the activity of the cholinergic neuronal markers hemicholinium-sensitive, high-affinity choline uptake (HACU) and choline acetyltransferase (ChAT) in the brain of aging Fisher 344 male rats. We now report the results from correlative experiments measuring the release of endogenous acetylcholine from slices of frontal cortex, hippocampus and striatum using the chemiluminescence method of Israel and Lesbats (1982). In 24 month old rats, a substantial decrease in ChAT (31%) is measured in striatum, and HACU is decreased in frontal cortex (30%) and hippocampus (23%) compared to 4 month controls. ACh release is decreased in frontal cortex (12%) and striatum (23%) compared to 4 month controls with no change in the hippocampus. Treatment of aged rats with NGF for 2 weeks results in supranormal stimulations of ChAT activity in frontal cortex, hippocampus, and striatum, 130% compared to young controls. HACU activity in NGF-treated aged rats is increased in frontal cortex and striatum similar to ChAT, but there is no effect of NGF on HACU in the aged hippocampus. ACh release is increased only in the striatum of NGF-treated aged rats, 130% compared to untreated aged rats. These data indicate an age-related differential regulation of ChAT, HACU, and ACh release between specific brain areas. Although NGF can stimulate the expression of these cholinergic markers in aged rat brain, there is an age-associated differential sensitivity of particular brain regions to exogenous NGF. ACh release does not correlate necessarily with changes in HACU activity.

CR 212 A POSSIBLE NEUROTROPHIC ROLE FOR IGF-I AND IGF-II ON MOTONEURONS, Jeffrey B. Wood, James McManaman, Department of Neurology, Program in Neuroscience, Baylor College of Medicine, Houston, TX 77030

It is well accepted that target derived trophic molecules are necessary for the normal development and maintenance of embryonic neurons. Recent experiments have suggested that insulin-like growth factors (IGFs) may affect the properties of CNS-derived cells. To test the possibility that IGFs may also act as neurotrophic factors for spinal motoneurons, we investigated the effects of IGFs I and II on cultured embryonic spinal cord cells and examined the binding of IGF-I to embyronic motoneurons by slide-mounted autoradiography. Both IGF-I and IGF-II are potent stimulators of cholinergic development in E14 rat spinal cord cultures. Their effects are dose dependent and inversely proportional to the cell density of the cultures. Specific binding of IGF-I to transverse sections of E14 rat spinal cord was assayed by slide mounted autoradiography. The results of these experiments demonstrate that IGF-I specifically binds to the ventrolateral portion of the embryonic spinal cord, a position normally held by the developing motoneurons. Though further characterization is required, we believe that this evidence suggests that the IGFs may play a role in the development, differentiation, and survival of motoneurons.

Neurotrophic Factors

CR 213 NEUROTROPHIC FACTOR FOR SEROTONERGIC NEURONS IS EXTRACTED FROM THE DENERVATED TARGET BRAIN: I. INJURY FACTOR Feng C. Zhou and James Murphy, and Efrain C. Azmitia, Dept. of Anatomy, Indiana University, Indianapolis, IN 46202 and Dept. of Biology, New York University, NYC, 10003

Serotonergic target brain tissue before innervation or after denervation were found to effectively stimulate the sprouting of serotonergic neurons in the brain --- (a) 5,7-dihydroxytryptophan (5,7-DHT) lesion in the hippocampus or spinal cord (Zhou and Azmitia, 1986; Wiklund and Bjorklund, 1980) or (b) placement of fetal striatal or hippocampal tissue (Zhou and Buchwald, 1989, Azmitia et al., 1982) triggers collateral sprouting or regenerative sprouting or 5-HT fibers.

In order to detect the neurotrophic signal released in the injured hippocampus in situ, the growth of grafted embryonic 5-HT neurons in normal and in injured hippocampus was used as an in vivo assay. Higher elevations of 5-HT content, affinity uptake, and 5-HT fiber density were observed in the injured hippocampus. To further detect the neurotrophic factor from the denervated hippocampus, an high-speed supernatent were extracted from injured hippocampus. This extract was effective in increasing the 5-HT content, high affinity uptake, fiber-growth, and survival of the grafted 5-HT neurons. The extract when placed with grafted 5-HT neurons increased the 5-HT content of grafted 5-HT neurons in the normal hippocampus. Furthermore, the grafted 5-HT neurons and trophic extracts were tested in the cerebellum, a brain region with the least 5-HT innervation. We found that grafted 5-HT neurons seldom survived in this brain region. The extracts co-transplanted with embryonic 5-HT neurons greatly increased the survival rate of the grafted 5-HT neurons, the 5-HT content, and the density of the 5-HT fibers in the cerebellum.

This data suggest that (a) the chemical injury which denervated 5-HT fibers turned the target brain tissue into a trophic environment; (b) the trophic environment could be a result of releasing of neurotrophic factor which favor the 5-HT neurons; (c) the neurotrophic factor in the extracts promoted serotonergic neurons in three levels--survival, maturation and fiber extension.

Disease and Regeneration

CR 300 PDGF'S AND FGF'S ARE POTENT MITOGENS FOR RAT SCHWANN CELLS, REQUIRING ELEVATED INTRACELLULAR cAMP FOR ACTIVITY, John B. Davis and Paul Stroobant, Ludwig Institute for Cancer Research, 91 Riding House Street, London W1P 8BT, England.

Rat sciatic nerve Schwann cells respond to a limited range of mitogens in vitro, including glial growth factor, TGF-ß1 and -ß2, some neuronal cell-surface associated factors, and to agents such as cholera toxin and forskolin, which raise intracellular levels of cAMP. Except for a neurite cell surface mitogen, these responses require the presence of serum, which exhibits little or no activity in the absence of other factors. Porcine-PDGF, human-PDGF, basic-FGF and acidic-FGF are nonmitogenic in the presence of 10%FCS alone. We show that they are, however, highly mitogenic in the presence of agents that elevate intracellular cAMP, such as forskolin and cholera toxin, increasing DNA synthesis, cell number, density, and affecting morphology. pPDGF gave half-maximal stimulation at 15pM, and hPDGF an equivalent response at 1nM. Basic-FGF was half-maximal at 5pM, acidic-FGF at 1nM. Experiments with recombinant PDGF-BB and PDGF-AA suggest that response to PDGF is dependent upon the PDGF-B type receptor.

Recognition of PDGF's and FGF's as mitogens for Schwann cells has implications for the study of Schwann cell proliferation in the development and regeneration of nerves. This is particularly so with respect to the role of cAMP raising mitogens located on the neurite cell surface, serum-derived PDGF, and involvement of macrophage-derived growth factors.

CR 301 **FOCAL SYNAPTIC LOSSES IN THE HIPPOCAMPAL COMPLEX IN ALZHEIMER'S DISEASE,** **L.J. DeGennaro, J.E. Hamos and D.A. Drachman, Dept of Neurology, Un. of Massachusetts, Worcester, MA 01655**

We have used synapsin I and synaptophysin -specific antibodies to assess changes in patterns of synaptic terminals in postmortem human brain tissue. Tissue from control subjects was compared with that of patients diagnosed as having Alzheimer's Disease. The results can be summarized as follows: 1) Histochemical staining by synapsin I and synaptophysin -specific antibodies revealed a focal loss of immunoreactivity in the outer half of the molecular layer of the dentate gyrus in demented patients. A similar loss is not apparent in age-matched controls; 2) These changes are independent of the presence of neuritic plaques or neurofibrillary tangles; 3) The focal reduction in staining is not simply a phenomenon of terminal stages of the disease as it occurs in at least one patient who exhibited only early signs of dementia. We hypothesize that this focal loss of synapsin I and synaptophysin immunoreactivity may reflect the loss of synaptic terminals and may represent an early event in the pathology of dementia. Our data suggest that changes in synapsin I gene activity might be used as a neuron-specific monitor of the timing and extent of neuronal pathology in Alzheimer's disease.

Neurotrophic Factors

CR 302 A MAJOR AMYLOID RNA IN HUMAN BRAIN IS SPECIES SPECIFIC AND MAY ENCODE A NOVEL SOLUBLE PROTEIN.

J. Steven Jacobsen, Arthur J. Blume and Michael P. Vitek, Molecular Neurobiology Group, CNS Research, Lederle Laboratories Division, American Cyanamid Company, Pearl River, NY 10965

Using an S1 nuclease protection assay, we have identified a new form of Amyloid Peptide Precursor (APP) RNA in human brain which does not comigrate with the 695, 751 and 770 forms. The sequence of a 1.6 Kbp cDNA clone corresponding to this new form predicts the synthesis of a 365 aa protein that is similar to the amino-terminal end of APP 770 but lacks the Beta-Amyloid-Peptide and any hydrophobic transmembrane spanning regions. Theses features suggest that an Amyloid Related Protein of 365 aa (ARP 365) is soluble, contains a Kunitz protease inhibitor domain and a novel carboxy-terminus.

S1 analysis of ARP 365 RNA shows it to be more abundant than APP 770, APP 751 and ARP 563, less abundant than APP 695 and virtually absent from mouse and rat brain RNAs. We have confirmed our S1 analysis results by RPC analysis which uses Polymerase Chain reaction to amplify cDNAs resulting from Reverse transcription of target RNAs. With RPC, we have found that human brain, but not mouse or rat brain RNAs, program the synthesis of a 420bp DNA corresponding to an ARP 365 transcript. Northern blots of human brain and HL60 RNAs probed with ARP 365 specific riboprobes yield complex patterns. These data suggest that humans express ARP 365 RNA and perhaps its cognate protein which have not been detected in rodents. Thus, amyloid plaques, a hallmark lesion of Alzheimer's disease brains which have not been reported in mouse and rat brains may require the participation of ARP 365 for plaque formation.

CR 303 NEURONAL PLASTICITY AND ASTROCYTIC REACTION IN DOWN SYNDROME AND ALZHEIMER DISEASE,

Ole Steen Jørgensen[1], B.W.L. Brooksbank[2] and R. Balázs[3].
[1]Psychochemistry Institute, Univ. Copenhagen, Denmark, [2]MRC Develop. Neurobiol. Unit, London, U.K. and [3]Netherlands Institute for Brain Research, Amsterdam, The Netherlands.

Proteins relatively enriched in neurons (NCAM and D3-protein) or in glia (glutamine synthetase (GS), glial fibrillary acidic protein (GFAP), and S100) were measured by quantitative immunochemical methods in autopsy samples of the cerebral cortex of subjects with Alzheimer disease (AD) or adults with Down syndrome (DS). The biochemical make-up of astrocytes was differentially affected: in both the frontal and temporal cortex of DS cases the specific concentration of GS was unaltered, while that of S100 and GFAP was markedly elevated (res ectively about 260% and 690% of control values). In the AD frontal cortex the estimates for GS were normal, while S100 and GFAP were about 180% and 230% of control. The observations suggested that the pathological changes involve an astrocytic reaction that is more marked in DS than in AD. In DS the increase in S100 could be explained, in part, by a gene dosage effect (as the S100β gene is located on chromosome 21, which is trisomic in DS), and in part by reactive gliosis. The neuronal markers were also differentially affected. In comparison with appropriate controls, the concentration of D3-protein in frontal cortex was decreased by 24% in DS and by 14% in AD, whereas NCAM levels were not significantly affected. The ratio of NCAM to D3-protein was increased 32% and 8.5%, in DS or AD respectively. These observations are consistent with the view that the destruction of mature neuronal structures (as marked by the D3-protein) coincides with the formation of new neuronal membranes (as indicated by NCAM).

CR 304 INDUCTION OF Tα1 α-TUBULIN mRNA DURING DEVELOPMENT, REGENERATION AND SPROUTING OF SYMPATHETIC NEURONS.

T.C. Mathew, R.B. Campenot, and F.D. Miller, Department of Anatomy and Cell Biology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Regulation of Tα1 α-tubulin mRNA, the major embryonic α-tubulin mRNA, was studied in sympathetic neurons of the superior cervical ganglia during development, regeneration and sprouting. Tα1 α-tubulin mRNA is highly enriched in the developing superior cervical ganglia (SCG), with expression being correlated with neuronal process extension. Transection of the internal carotid nerve either by unilateral enucleation of the eye (long cut) or by cutting the nerve close to the ganglion (short cut) leads to an upregulation of Tα1 α-tubulin mRNA in the regenerating neurons. Experiments comparing Tα1 mRNA in long cut neurons and short cut neurons in the same ganglia demonstrate that Tα1 mRNA reaches peak levels that are significantly higher in the short cut neurons. Tα1 α-tubulin mRNA levels also increase in the contralateral, uninjured ganglia following a short cut but not a long cut. This increase in the contralateral SCG coincides with the sprouting of mature, uninjured sympathetic neurons, as demonstrated by tyrosine hydroxylase immunoreactivity in the pineal gland. These data suggest that induction of Tα1 α-tubulin mRNA during collateral sprouting may be due to the increased availability of trophic factors such as NGF from bilaterally-innervated target organs. Support for this hypothesis derives from experiments demonstrating that Tα1 mRNA is abundantly expressed in primary cultures of sympathetic neurons and is regulated by NGF is a dose-dependent fashion in these cultures. Furthermore, Tα1 α-tubulin mRNA can be increased in sympathetic neurons *in vivo* by systemic administration of NGF. We are currently investigating the intrinsic and extrinsic signals involved in regulating this growth-associated mRNA during the regeneration and sprouting of mature, sympathetic neurons.

Neurotrophic Factors

CR 305 EXPRESSION OF TAU AND BETA-AMYLOID PRECURSOR PROTEINS IN BACULOVIRUS. Lisa McConlogue, Jeroen Knops, Athena Neurosciences, South San Francisco, CA 94080.
Tau protein is a component of Alzheimer's disease tangles, and the beta-amyloid precursor protein (βAPP) is the precursor of the amyloid deposited in Alzheimer's disease plaques. We have expressed 3 and 4 repeat forms of human tau protein and 695 and 751 forms of the human βAPP in the baculovirus system. Multiple forms of tau protein are produced in the infected insect cells, at a level of 50 mg/L. The βAPP is produced in these cells as both a cellular full length form and a truncated secreted form. The secreted form lacks an antigen from the carboxy-terminal region of this protein. The full length form is produced at 50 mg/L and the secreted form at 15 mg/L. This system provides us with large amounts of these proteins that we will use to develop in vitro models of Alzheimer's disease plaque and tangle formation.

CR 306 IDENTIFICATION OF CELLULAR PROTEINS BINDING TO THE SCRAPIE PRION PROTEIN. Bruno Oesch, Dave B. Teplow, Neil Stahl, Leroy E. Hood and Stanley B. Prusiner, UC San Francisco, CA 94143-0518 and California Inst. of Technology, Pasadena, CA
The scrapie prion protein (PrP^{Sc}) is part of the infectious particle causing scrapie and other prion diseases. We have employed ligand blots to identify two major proteins binding to PrP with molecular weights of 45,000 and 110,000 denominated PrP ligands (Pli 45, Pli 110). As a probe we used radioiodinated PrP 27-30 which is the protease-resistant core of PrP^{Sc}. Unlabeled PrP 27-30 but not other proteins competed with the radiolabelled probe. Pli 45 was more abundant in scrapie-infected than in normal brain. The dissociation rate constant of the Pli 45/PrP 27-30 complex was $3x10^{-6}$ s^{-1}. Subsequent purification and sequencing of Pli 45 revealed a very high homology to murine glial fibrillary acid protein (GFAP). This was corroborated by immunological crossreaction of anti-Pli 45 antibodies with recombinant GFAP indicating that astrocytes which produce GFAP may be involved in scrapie and other prion diseases. It has been observed that infectious particle (which most probably contain PrP^{Sc}) copurify with GFAP. Pli 110 was found in scrapie-infected and normal brain as well as other tissues. In view of the homology of PrP with a factor inducing acetylcholine receptors in chicken muscle (ARIA; Harris et al., Soc. Neurosci. Abstr. 15, 70.7, 1989) Pli 110 may be related to the normal function of PrP in the central and peripheral nervous system.

CR 307 NON-NEURONAL GENE EXPRESSION FOLLOWING SCIATIC NERVE TRANSECTION.
Jean G. Toma, A. Acheson, P.A. Barker, S. Pareek, T.C. Mathew, R.A. Murphy, and F.D. Miller. Department of Anatomy and Cell Biology, University of Alberta, Edmonton, Alberta, T6G 2H7.

Neurons in the peripheral nervous system are able to successfully regenerate following axonal injury. Non-neuronal cells in the local environment encountered by the injured axons play an essential role during regeneration. The molecular mechanisms underlying this phenomenon, however, have yet to be characterized. To begin to approach this problem, we have investigated the amount and localization of epidermal growth factor receptor (EGFR) and nerve growth factor receptor (NGFR) in the transected sciatic nerve. *In situ* hybridization analysis demonstrated an increase in the expression of NGFR and EGFR mRNAs within 3 hours of a transection. This increase was confined to fibroblasts of the perineurial sheath of both proximal and distal nerve stumps. By 36 hours, elevated levels of EGFR and NGFR were detectable over cells within both the epineurium and perineurium, and were particularly high at the cut sites. The increases in the proximal segment were restricted to the region immediately adjacent to the lesion, whereas those in the distal segment extended throughout the whole length of the perineurial sheath. Immunostaining of adjacent sections with an antibody to NGFR (IgG 192) confirmed the increases observed by *in situ* hybridization, and demonstrated that there was a proximal- distal gradients of NGFR in the proximal nerve stump. In addition to the observed changes in abundance of trophic factor receptors, the amount of histone H3.3 mRNA, a partially replication-independent histone varient, increased dramatically in cells of the perineurial sheath; this increase may reflect cell division. Thus, there are rapid and dramatic changes in both EGFR and NGFR in cells of the perineurium and epineurium (probably fibroblasts) following axonal injury.

Neurotrophic Factors

Glial Cells

CR 400 SERUM-FREE CULTURE AND DIFFERENTIATION OF ASTROCYTE PRECURSORS,
D. Barnes, Y. Sakai and D. Loo, Dept. of Biochemistry and Biophysics, Environmental Health Sciences Center, Oregon State Univ., Corvallis, OR 97331
Serum-free mouse embryo (SFME) cells derived in nutrient medium supplemented with insulin, transferrin, epidermal growth factor, high density lipoprotein and fibronectin do not exhibit growth crisis or gross chromosomal abnormalities and are reversibly growth inhibited by serum (Loo et al., Science 236, 200). Exposure of SFME cells to 10% serum or 10 ng/ml transforming growth factor beta (TGF beta) for 24 hours induced expression of glial fibrillary acidic protein (GFAP). Platelet-free plasma also induced GFAP, and the plasma activity was destroyed by boiling, suggesting that factors in serum other than TGF beta may be involved in GFAP induction. No GFAP induction was observed with fibroblast growth factor (FGF) or platelet-derived growth factor. Induction of GFAP in SFME cells was not correlated with growth inhibition. Hydrocortisone was growth inhibitory for SFME cells but did not induce GFAP expression, and SFME cells transformed by ras grew in serum-containing medium but expressed GFAP. Cells with responses similar to SFME cells were derived from adult mouse brain and embryonic human brain in the SFME medium supplemented further with 10 ng/ml FGF and 1 ug/ml heparin. These results describe a precise system for manipulation of astrocyte differentiation, distinguish several mediators of this differentiation, and suggest that undifferentiated astrocyte precursors may exist in adult brain.

CR 401 *IN SITU* HYBRIDIZATION OF BASIC FGF mRNA AFTER LESION OF THE CORTEX: POSSIBLE INVOLVEMENT OF MACROPHAGES AND ASTROCYTES.
Sally Frautschy, *Patricia Walicke, Andrew Baird, Molecular and Cellular Growth Biology Whittier Institute La Jolla CA 92037, *Dept. Neurosciences, University of California, San Diego, La Jolla CA. 92037
The experiments described here were conducted to determine the effect of cortical lesions on the expression of basic FGF mRNA and to identify the cell-types that immunolocalize basic FGF in male Sprague-Dawley rats (200 g). A 1.8 x 2.5 mm region of the right cingulate and frontal cortex and corpus callosum was aspirated (0-1.8 AP, 0-2.5 ML posterior to bregma), and animals were perfused at 4 h, 1d , 2d, 3d 7d, and 14d (n=3). This was compared to the contralateral side and to the cortex of 3 rats who were not lesioned. Using a rat cDNA clone for basic FGF, the antisense strand was labeled with [35S] and hybridized to 25μm brain sections. The hybridization was evaluated by exposure of the slides to Kodak X-AR film and autoradiographic emulsion. Double immunoflourescence was performed on adjacent sections by labeling macrophages or astrocytes with monoclonal antibodies against OX-42 (1:75, Serotec) or glial fibrillary protein (GFAP, 1:550, Sigma) respectively. These sections were double-labeled with antibodies against basic FGF 1-24 (1:400). Results of *in situ* hybridization demonstrated that the expression of basic FGF mRNA at 4 h persisted up to 14d in the region surrounding the lesion and dramatically differed from the absence of basic FGF mRNA in the corresponding sections of control animals or the contralateral side. Results of immunocytochemistry showed that at 1 and 2d, the cells that immunolocalized basic FGF resembled monocytes but were OX-42 negative. At 3d and 7d, many of the cells which resembled macrophages (not microglia) labeled with OX-42 and contained basic FGF. Immunoreactve basic FGF was present in astrocytes at 7 and 14d. These results demonstrate that an increased synthesis of basic FGF is an early but persisting response to injury in the rat brain. Whether the presence of basic FGF initially in macrophages and later in astrocytes is due to the synthesis of basic FGF by these cells or due to utilization of basic FGF by these cells is presently under investigation.

CR 402 REDUCED EXPRESSION OF MHC CLASS II MOLECULES IN A MURINE OLIGODENDROGLIOMA PERSISTENTLY INFECTED WITH THE MURINE CORONAVIRUS, JHMV.
Wendy Gilmore, John Fleming*, Molly Moloney, Elaine Pan and David Richardson, Department of Neurology, USC School of Medicine, 2025 Zonal Ave., Los Angeles, CA 90033 and *Department of Neurology, University of Wisconsin Medical School, Madison, WI 53792.
The G26-20 glioma is an immature murine glial tumor cell line bearing several characteristics of an oligodendrocyte, including CNPase activity and gene expression, and cerebroside and sulfatide synthesis. Using indirect immunofluorescence and FACS analyses, our laboratory has determined that G26-20 cells also express MHC Class I and II molecules in response to recombinant murine gamma-interferon (g-IFN; 1-1000 u/ml; 24-96 hr). We have succeeded in maintaining more than 80 passages of G26-20 cells infected with the murine coronavirus, JHMV, which causes demyelination in chronically infected mice and rats. The persistently infected cells yield virus titers varying from 10^1 to 10^5 pfu/ml with successive passages. Virus-induced cell fusion affects up to 30% of the cells, with immunoperoxidase and *in situ* hybridization studies showing that approximately 50% of the cells contain viral RNA in the presence or absence of viral proteins. MHC Class II expression is reduced by 40-64% in JHMV-infected cells relative to uninfected cells following *in vitro* treatment with g-IFN. By contrast, MHC Class I expression is not altered. The data indicate that JHMV infection selectively alters cellular gene expression in G26-20 cells, and suggest that they are a useful model for the investigation of mechanisms of virus-induced neurological disease.

CR 403 NEURONAL AND GLIAL CELL LINES PRODUCE MULTIPLE GROWTH FACTORS FOR CELLS OF THE O2A LINEAGE. Joel M. Levine, Frances A. Stincone and Behzad Maghsoudhlou, Dept. of Neurobiology and Behavior, S.U.N.Y. at Stony Brook, Stony Brook,NY,11794
Oligodendrocytes and some astrocytes develop from a common bipotential precursor cell termed an O2A progenitor cell. The development and differentiation of O2A progenitor cells can be regulated by environmental factors such as PDGF, bFGF and CNTF. To begin to characterize factors capable of influencing the differentiation of glial precursor cells, we have investigated the responses of O2A progenitor cells to factors secreted by neuronal and glial cell lines. Post-natal day 5 rat optic nerve cells were grown for 3 days in serum-free medium conditioned by the cell lines (CM) and the development of the cultures was monitored by immunofluorescent staining to identify the cell types present and thymidine autoradiography to identify dividing cells. All of the glial (B82,B92,B49,C6 and A172) and neuronal cell lines tested (B103,B35,B50,B65 and N2A) secreted mitogens for progenitor cells. The effects of these CMs could be partially mimicked by adding PDGF to control cultures. These cell lines also contained mRNAs encoding the PDGF-A chain. Many of the CMs stimulated thymidine uptake in NIH3T3 cells which respond to PDGF and in CHO cells which lack a PDGF receptor. Medium conditioned by B92 cells promoted the development of type 2 astrocytes in the serum-free cultures. Consistent with the hypothesis that B92 cells secrete a CNTF-like substance, B92 CM supported the growth of E8 chick ciliary neurons. These data demonstrate that both neuronal and glial cell lines are a rich source of trophic activities that can influence the development of O2A progenitor cells.

CR 404 ANGIOTENSIN II BINDS TO SPECIFIC CELL SURFACE RECEPTORS AND STIMULATES SECRETION OF A M_r 55,000 PROTEIN BY ASTROCYTES CULTURED FROM 21-DAY-OLD RAT BRAIN: John A. Olson, Susan Ogilvie, Kathleen T. Shiverick, and Mohan K. Raizada, Departments of Pharmacology and Therapeutics and Physiology, University of Florida, College of Medicine, Gainesville, FL 32610.

Angiotensin II (Ang-II) has been documented to exert physiological actions on the brain pertinent to the central control of blood pressure. It is largely assumed that these effects of Ang-II are mediated by its actions on neuronal cells since these cells have been shown to express Ang-II specific receptors. Previous observations by our group demonstrating that astroglial cells also express functional Ang-II receptors has prompted us to reevaluate the mechanism of Ang-II actions in the CNS. We postulate that astroglial Ang-II receptors may be involved in the transmission of chemical signals between these cells to other CNS cells i.e. neurons, oligodendrocytes and the endothelium. This hypothesis is consistent with established anatomical associations among these cells. Astroglia in primary culture from the brain of 21-day-old rats have been established to study Ang-II specific receptors and Ang-II action. Binding experiments using ^{125}I-Ang-II, demonstrate that astroglia from 21-day-old brain possess specific, high affinity (Kd = 0.5 nM) and saturable (Bmax 80 fmoles/mg protein) Ang-II specific receptors. 2-D-SDS-PAGE analysis of [^{35}S] methonine-labelled secretory proteins was also performed to assess potential Ang-II effects on intercellular communication. Ang-II stimulated the accumulation of a secretory protein (pI M_r 55,000 5.5-6.0) from these cultures, an effect blocked by Ang-II receptor antagonist, (Sar^1,Ile^8) Ang-II. These observations indicate that: (i) astroglia from mature brain can be established in primary culture; (ii) Ang-II specific receptors are expressed in these cells; and (iii) activation of these receptors by Ang-II stimulates secretion of a specific M_r 55,000 protein. The role of this secretory protein as a chemical messenger remains to be elucidated.

CR 405 AGONIST DEPENDENT DIFFERENCES IN MOLECULAR SPECIES OF STIMULATED DIGLYCERIDES, Melissa S. Pessin, Joseph G. Altin, Ralph A. Bradshaw, and Daniel M. Raben, Departments of Neuroscience and Physiology, The Johns Hopkins University School of Medicine, Baltimore, MD 20205, and Department of Biological Chemistry, California College of Medicine University of California, Irvine, CA 92717
The role of cellular 1,2-diglycerides in transmembrane signalling is now well established. We have previously described a method for analyzing the molecular species of cellular DGs and phospholipids (Pessin, M.S. and Raben, D.M. (1989) J. Biol. Chem., 264, 8729-38). We have applied these techniques to two different systems: 1) mitogenic stimulation of quiescent fibroblasts; and 2) neurotransmitter and differentiating factors in PC12 cells. Comparison of these different diglyceride and phospholipid molecular species profiles in both cell types, indicates that during a biphasic generation of diglycerides, the first peak arises largely from phosphoinositide hydrolysis. In contrast, the second peak and monophasic peaks all arise primarily, if not exclusively, from phosphatidylcholine hydrolysis. Interestingly, within a single cell type, significant differences exist between each of the diglyceride molecular species profiles generated by phosphatidylcholine hydrolysis. This result can be explained either by 1) the stimulation of an agonist-selective metabolism of diglycerides, or 2) the existence of agonist-specific phosphatidylcholine pools.

Neurotrophic Factors

CR 406 NEUROPEPTIDE GENE EXPRESSION IN CULTURED ASTROCYTES: BRAIN REGION AND GENE SPECIFICITY AND DEVELOPMENTAL TIME COURSE. J.P. Schwartz, H.Shinoda, and A.M.Marini, Clinical Neuroscience Branch, NINDS, NIH, Bethesda, MD 20892. Astrocytes exhibit many neuronal characteristics, such as neurotransmitter receptors, ion channels and neurotransmitter uptake systems. We have shown [Science 245: 415 (1989)] that cultured astrocytes express certain neuropeptide genes, with a specificity shown for both the gene expressed and the brain region from which the cells were prepared. Somatostatin (SS) mRNA and peptide are present only in cerebellar astrocytes whereas proenkephalin (PE) mRNA and enkephalin peptides are present in astrocytes of cortex, cerebellum and striatum. Cholecystokinin mRNA is not expressed in any of the cells. Exposure of cultured astrocytes to forskolin, which elevates cyclic AMP, increases both PE mRNA and enkephalins. Developmentally, the content of PE mRNA remains fairly constant but met-enkephalin is highest in cells prepared from embryonic day 20 (E20) or postnatal day 3 (D3) animals, with significantly less met-enkephalin in D8 or adult astrocytes. In contrast, both SS mRNA and SS peptide decrease continuously in cerebellar astrocytes from E20 to adult, showing a parallelism to what is seen in intact cerebellum and suggesting that the SS in the cerebellum may be present only in astrocytes. These data suggest the possibility that astrocyte-derived peptides may play a trophic function early in CNS development.

CR 407 RESCUE OF AXOTOMIZED BASAL FOREBRAIN CHOLINERGIC NEURONS AFTER IMPLANTATION OF GENETICALLY MODIFIED, NGF-PRODUCING FIBROBLAST CELLS,
C. J. Wetmore[1], P. Ernfors[2], H. Persson[2], T. Ebendal[3], I. Strömberg[1], L. Olson[1]. Depts. of [1]Histology & Neurobiology and [2]Molecular Neurobiology, Karolinska Institute, Stockholm, Sweden; Biomedicum, [3]Uppsala Univ., S-751 Uppsala, Sweden.

Mouse 3T3 fibroblasts, genetically modified by transfection with several hundred copies of the rat NGF gene, secrete high levels of biologically active NGF. Grafts of the genetically-modified, NGF-producing cells rescued axotomized basal forebrain cholinergic neurons and significantly reduced cholinergic cell death in the medial septum as compared to rats treated with the non-transfected parental 3T3 cells. We suggest that implantation of genetically modified cells producing NGF may have therapeutic applications in rescuing damaged central cholinergic neurons in senile dementia of the Alzheimer's type as well as in providing trophic support for grafts for treatment in Parkinson's disease.

CR 408 EXPRESSION OF ACIDIC-FIBROBLAST GROWTH FACTOR (a-FGF) mRNA IN RAT BRAIN. Barbara J. Wilcox, Jeffery L. Twiss and James R. Unnerstall, Department of Neurology and The Alzheimer Center, Case Western Reserve University School of Medicine, Cleveland, Ohio, 44106. Using a 36mer oligonucleotide probe corresponding to amino acids 26-37 of bovine aFGF, we have localized mRNA for aFGF in developing and adult rat brain by *in situ* hybridization histochemistry. Nine time points in brain development from embryonic day 17 to postnatal day 21 were selected. Results from hybridization of [35S]probe to slide-mounted parasagittal sections of brain tissue from earlier time points (E17-E20) showed a generalized distribution of labeling throughout the brain with enrichments of labeling found in the developing cortical plate. At later time points (P1-P21), a distinct pattern of labeling to the pyramidal cell layer of the hippocampus was observed. During the second postnatal week, discrete localization of labeling also appeared in the developing granule cell layer of the dentate gyrus. Examination of autoradiograms under high magnification confirmed this discrete localization of autoradiographic grains to pyramidal and dentate granule neurons. The appearance of aFGF mRNA expression in the hippocampal formation and dentate gyrus corresponds with the maturation and migration of pyramidal and granule cells to their adult location. In the adult rat brain, a discrete pattern of labeling was found in the pyramidal cell layer of the hippocampus and the granule cell layer of the dentate gyrus. No labeling was observed when serial sections were hybridized with a corresponding sense probe. Northern blot analysis revealed labeling of a single band of 4.8 kb which is in agreement with the reported size for aFGF message in brain. These data suggest that pyramidal and dentate neurons in the hippocampal formation may be a source of aFGF in brain and that this neurotrophic factor may play a significant role in brain development.