THE MOLECULAR AND CELLULAR BASIS OF HUMAN NEURODEGENERATIVE DISEASE

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Plenary Sessions *Page*

Poster Sessions

Developmental and Experimental Models of Cell Death in the Nervous System

D3-001 EXCITOTOXICITY IN VITRO: NECROSIS VS. APOPTOSIS, Dennis W. Choi, Center for the Study of Nervous System Injury and Department of Neurology, Washington University School of Medicine, St. Louis, MO 63110.

We have previously described two forms of excitotoxicity in primary murine cortical cell cultures: 1) rapidly-triggered excitotoxicity induced by brief intense stimulation of NMDA type glutamate receptors; and 2) slowly-triggered excitotoxicity induced by prolonged stimulation of AMPA/kainate type glutamate receptors. Rapidly-triggered excitotoxicity depends on extracellular Ca^{2+}, and is accompanied by substantial accumulation of $^{45}Ca^{2+}$ from the bathing medium, likely mediated in large part by NMDA receptor-gated channels. Slowly-triggered excitotoxicity may similarly reflect Ca^{2+} overload, initiated by a more gradual Ca^{2+} influx through voltage-gated Ca^{2+} channels or the Na^{+}/Ca^{2+} exchanger, as well as through a minority population of AMPA or kainate receptors that gate Ca^{2+}- permeable channels. Predictably, both forms of excitotoxic neuronal death appear to reflect necrosis.

Neurons in the same cultures exposed to transient oxygen-glucose deprivation developed marked acute cell body swelling followed by degeneration, consistent with rapidly-triggered excitotoxic necrosis. This death was substantially attenuated by addition of the NMDA antagonist MK-801 and the AMPA/kainate antagonist CNQX to the exposure medium, but not by addition of cycloheximide. If the duration of oxygen-glucose deprivation was extended to overcome the protective effect of glutamate antagonists, a different neuronal death resulted that was associated with cell body shrinkage and DNA fragmentation, and attenuated by cycloheximide. Present data thus raise the possibility that oxygen-glucose deprivation can induce in cortical neurons both excitotoxic necrosis, and apoptosis dependent on new macromolecule synthesis.

D3-002 *Abstract Withdrawn*

Influences of Trophic Factors in Cell Survival

D3-003 CYTOKINES IN NEURAL DEVELOPMENT AND INJURY, Paul H. Patterson, Biology Division, California Institute of Technology, Pasadena, CA 91125, USA.

As in the hematopoietic system, the enormous variety of phenotypes in the nervous system arises, in part, through the action of instructive differentiation signals. Neuronal culture assays have allowed the identification and cloning of several proteins that control the expression of phenotype-specific genes in developing neurons. A group of non-homologous cytokines induces the expression of the same set of neurotransmitter synthetic enzymes and neuropeptides in cultured sympathetic neurons. Several members of this neuropoietic cytokine group, which now includes CDF/LIF, CNTF, OSM and GPA, share a predicted secondary structure, as well as the use of a transducing receptor subunit in common with IL-6 and IL-11. The latter two cytokines also display a weak activity in the sympathetic neuron assay. In addition, we find that certain members of the TGFβ superfamily, activin A and BMP-2 and -6, each induce a distinct set of genes in sympathetic neurons, and those sets partially overlap with the spectrum of neuropeptides induced by the neuropoietic family. Neuronal responses to these various cytokines are differentially regulated by depolarization, such that neuronal activity can modulate phenotypic expression in a cytokine- and neuropeptide-specific manner.

To study the role of LIF (leukemia inhibitory factor)*in vivo*, we localized its mRNA and that of its receptor. LIF mRNA levels are developmentally modulated, and substantial differences are observed between tissues. Particularly high expression for both LIF and the LIFR is found in neurons of the visual system and brain stem of the adult rat. In sciatic nerve and sympathetic ganglia, the postnatal increase in receptor expression parallels expression of LIF. LIF mRNA levels increase dramatically after damage to peripheral nerve and ganglia, and a series of neuropeptide mRNAs are induced. Analysis of mice in which the LIF gene has been targeted by homologous recombination reveals that this neuropeptide induction is very weak in LIF^{-} mutant mice, demonstrating a key role for LIF in the injury response. LIF is also induced by injury to the normal, adult cortex. Since LIF expression is induced in a variety of inflammatory conditions, it is likely that this cytokine is active at the interface between the nervous and immune systems.

In addition, the brains of adult LIF^{-} mutant mice display distinct, atrophic changes in the visual cortex and dentate gyrus of the hippocampus. Similar changes are not observed in several other areas of cortex. Thus, LIF is likely to have a role in the development and/or maintenance of particular brain regions.

Patterson, PH and Nawa, H *Cell* 72:123 (1993); Rao, MS, et al. *Neuron* 11:1175 (1993); Fann, MJ and Patterson, PH *PNAS* 91:43 (1994), *J. Neurochem.* in press; Banner, LR and Patterson, PH *PNAS* 91:7109 (1994); Patterson, PH *PNAS* 91:7833 (1994).

D3-004 NEUROTROPHIC FACTORS AND THEIR MULTI-COMPONENT RECEPTOR COMPLEXES, George D. Yancopoulos, Regeneron Pharmaceuticals, INC., Tarrytown, NY 10591

Naturally occurring neuronal cell death accompanies normal embryonic development, whereas abnormal neuronal death is a key feature of a variety of neurodegenerative diseases. Neurotrophic factors were discovered for their ability to prevent neuronal cell death, and they elicit additional dramatic responses from neurons. Our work involves the discovery and characterization of neurotrophic factors, and the understanding of the receptor systems and signal transduction pathways utilized by these factors. I will talk about three distinct classes of neurotrophic factors: those related to Nerve Growth Factor (including Brain-Derived Neurotrophic Factor (BDNF), Neurotrophin-3 and Neurotrophin-4/5); those related to Ciliary Neurotrophic Factor (CNTF), including leukeima inhibitory factor (LIF) and interleukin-6 (IL6); and a new family of ligands we have recently discovered that utilize the EPH-family of receptor tyrosine kinases. I will focus on similarities and differences in the receptor systems and signaling systems used by these three classes of neurotrophic factors. I will also discuss synergies observed when different classes act simultaneously, as well as gene disruption studies that provide insights into the physiological roles of these factors.

Clinical and Genetic Aspects of Motor Neuron Disease

D3-005 SELECTIVE VULNERABILITY OF NEURONS WITH LEWY BODY-LIKE NEUROFILAMENT INCLUSIONS TO PREMATURE DEATH IN TRANSGENIC MICE. Virginia M.-Y. Lee, Pang-Hsien Tu and John Q. Trojanowski. Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-4283.

Amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) and diffuse Lewy body (LB) disease (DLBD) are characterized by the massive degeneration of selected populations of central nervous system (CNS) neurons many of which accumulate intraneuronal filamentous inclusions. These inclusions resemble aggregated masses of neurofilaments (NFs), and immunohistochemical studies implicate NF triplet proteins as the major building blocks of these abnormal filaments. In ALS, the inclusions are referred to as spheroids and they occur primarily in the proximal axons of spinal cord motor neurons. In PD and DLBD, these inclusions are termed LBs and they predominate in the perikarya of brain stem and cortical neurons. To address the role of NF-rich inclusions in the dysfunction and premature death of affected neurons, we examine the consequence of expressing a fusion protein comprised of the high molecular weight NF (NFH) protein and LacZ (NFHLacZ) in transgenic mice that accumulate prominent LB-like perikaryal aggregates of NFs in different neuronal populations of neurons of the CNS. We showed that only some populations of neurons died prematurely as a result of the accumulation of LB-like inclusions. For example, a remarkable decrease in the number of Purkinje neurons with prominent Bergmann gliosis was noted in 18 month-old transgenic mice and a mild but significant (20%) reduction of Purkinje cells was already noted in the 12 month-old transgenic mice. However, there was no significant neuronal loss in the hippocampal CA1 regions of the same mice. Thus, the selective loss of Purkinje cells was a progressive and age-dependent process. We further demonstrate by electron microscopy that the type and the average size of the aggregates in Purkinje cells was different from those in hippocampal neurons. Based on our observations, the NFHLacZ transgenic mice may provide a valuable animal model for studying neurodegenerative diseases associated with the accumulation of NF-rich LB inclusions and the selective loss of neurons.

Models of Motor Neuron Disease

D3-006 **IN VITRO MODELS OF MOTOR NEURON DEGENERATION,** Jeffrey D Rothstein. Johns Hopkins University, Dept. of Neurology, Baltimore, MD 21287.

ALS is a progressive neurologic disorder characterized by a slow loss of cortical and spinal cord motor neurons. The pathogenesis of sporadic ALS, the most common form, is unknown, but several observations suggest that glutamate could participate in selective motor neuron degeneration. Defects in synaptosomal high-affinity glutamate transport have been observed in ALS tissue. In organotypic spinal cord cultures, chronic loss of glutamate transport can produce a loss of motor neurons, and therefore could contribute to the disease. With the recent cloning of three glutamate transporters: EAAC1, GLT-1 and GLAST, it has become possible to determine if the loss of glutamate transport in ALS is subtype specific. C-terminal, anti-oligopeptide antibodies that were specific for each glutamate transporter have been used to localize glutamate transporter subtypes: EAAC1 is selective for neurons, GLT-1 is selective for astroglia, and GLAST can be found in certain neurons and astroglia. Brain and spinal cord tissue from ALS patients and appropriate control have revealed a modest loss (0-20% decrease from control) of EAAC1 protein in ALS, whereas there was no appreciable change in GLAST immunoreactive protein. However, GLT-1 protein was markedly decreased by 70% in ALS motor cortex, and in approximately 25% of the specimens examined (n=18) there was almost a complete loss of GLT-1 protein (80-99% decrease from control). Glial fibrillary acidic protein, which is selectively localized to astroglia, was not changed in ALS motor cortex. The loss of EAAC1 in motor cortex may be secondary to loss of cortical motor neurons. However, there is no loss of astroglia in ALS, and the dramatic abnormalities in GLT-1 in some patients could reflect a primary defect in GLT-1 protein. To study the role of each transporter subtype in glutamate excitotoxicity, antisense oligodeoxynucleotides to either GLT-1, GLAST, or EAAC1 were added to organotypic spinal cord cultures or administered chronically to animals. Initial studies indicate that both glial transporters can contribute to neurotoxicity and that loss of GLAST can produce a degenerative motor neuron-like disorder.

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Genetics of Alzheimer's Disease

D3-007 APOLIPOPROTEIN E4 AND E2 ARE SUSCEPTIBILITY GENES THAT AFFECT THE RATE OF ALZHEIMER DISEASE EXPRESSIVITY, Allen Roses[1], Ann Saunders[1], Elizabeth Corder[1], Neil Risch[2], Jonathan Haines[3], Margaret Pericak-Vance[1], Seol-Heui Han[1], Gillian Einstein[1], Christine Hulette[1], Donald Schmechel[1], Michel Goedert[4], Karl Weisgraber[5], David Huang[1], and Warren Strittmatter[1]. [1]Joseph and Kathleen Bryan Alzheimer's Disease Research Center, Duke University Medical Center, Durham, NC 27710. [2]Yale University, New Haven, CN, [3]Massachusetts General Hospital, Charlestown,MA, [4]MRC Laboratory of Molecular Biology, Hills Rd., Cambridge, UK, [5]Gladstone Institute of Cardiovascular Disease, University of California, San Francisco, CA.

Apolipoprotein E-e4 (APOE4,gene;apoE4,protein) is a susceptibility gene or risk factor for Alzheimer disease. The ApoE4 allele frequency is highly significantly increased in affected individuals in familial and sporadic late-onset Alzheimer disease. The inheritance of APOE4 is associated with increased risk of disease expression and a younger age of onset in the dose-dependent manner. The effect of the more uncommon allele, APOE2, is to decrease the risk and increase the age of onset compared to the common allele APOE3. Thus the effect of inheriting specific alleles of APOE is associated with regulating the age of disease expression of Alzheimer disease. ApoE antibodies stain neurons in autopsy brains from elderly controls, Alzheimer disease patients, and aged primates. In Alzheimer disease patients, many apoE immunoreactive neurons also contain immunoreactive neurofibrillary tangles. However, in situ hybridization studies suggest that apoE mRNA is not expressed in neurons. Immunoelectron microscopic studies have localized apoE in the cytoplasm of neurons. We have demonstrated that apoE3 binds non-phosphorylated tau in vitro to form an SDS-resistant complex, but there is no binding of apoE4 to tau. Neither apoE3 nor apoE4 bind to phosphorylated tau. ApoE3 binding occurs in the microtubule binding repeat domains of the tau molecule. Initial studies with isolated repeat domain peptides show that the binding of ApoE3 is not dependent on the formation of disulfide bonds. Evidence for a model to explain the rate of disease expression as a function of APOE genotype where apoE3 and apoE2 play a protective role by sequestering free tau and facilitating microtubule stabilization is supported by neuronal degeneration studies of APOE knock-out mice.

D3-008 THE CHROMOSOME 14 ALZHEIMER'S DISEASE LOCUS. Gerard D. Schellenberg[1], Vikram Sharma[1], Parvoneh Poorkaj[1], Fuki Hisama[2], Ellen M. Wijsman[1], Sherman M. Weissman[2], Thomas D. Bird[1,3]. [1]University of Washington, Seattle, WA 98195; [2]Yale University, New Haven CT 06520; [3]Veteran's Affairs Medical Center, Seattle, WA 98108-1532.

Early-onset autosomal dominant familial Alzheimer's disease (AD) is genetically heterogeneous. In some families, mutations in the APP gene are responsible for the disease. In others, an as yet unidentified locus on chromosome 14q24.3 is responsible for the disease. In still others, notably the Volga German kindreds, the responsible locus remains unmapped. We are attempting to identify the chromosome 14 locus, referred to as AD3, by positional cloning methods. The candidate region has been defined genetically by identifying obligate recombinants in kindreds in which AD is clearly the result of the AD3 locus; definitive flanking markers are D14S61 and D14S298 as defined by obligate recombinants between affected subjects. These markers are 9 cM apart. The entire critical region has been cloned as yeast artificial chromosome (YAC) clones. This contig consists of 40 YACs of varying sizes. This contig is part of a larger YAC contig spanning *D14S61* to *D14S63* consisting of 74 clones. In the *AD3* candidate region, all YACs are linked by 1 or more sequence-tagged sites. These STS markers consist of 16 polymorphic markers, 13 expressed sequenced tagged sites (ESTs), and 5 published cloned genes. The STSs are also being used to identify cosmids from a chromosome 14-specific library arrayed for PCR screening. cDNA selection is being used to identify genes in the AD3 region. By this procedure, YAC DNA is hybridized to a mixture of 6 cDNA libraries, and sequences specifically hybridizing to the YACs are cloned using a λ-vector. The resulting inserts are then sequenced and analyzed for homology to known genes using BLAST. The resulting clones are typically 150 to 250 bp in length and represent only partial cDNA clones. These clones are then being used to screen cDNA libraries for full-length cDNA clones. To date, 108 short inserts have been sequenced representing 64 unique sequences. Twelve of these clones have been used to screen cDNA libraries and longer clones have been obtained. When full-length clones are identified, RT-PCR and direct sequencing will be used screen patient DNA for the AD3 mutations.

D3-009 MOLECULAR GENETIC STRATEGIES FOR ISOLATING THE MAJOR EARLY-ONSET FAD GENE ON CHROMOSOME 14, Rudolph E.Tanzi, Donna M. Romano, Warren Pettingell, Paul Jondro, Steven Schmidt, Annette C.Crowley, Marc Paradis, Jill Murrell, Alan Buckler, Wilma Wasco. Laboratory of Genetics and Aging, Mass. General Hospital, Harvard Medical School, Charlestown, MA 02129

Genetic studies of kindreds displaying evidence for familial AD (FAD) have led to the localization of gene defects responsible for this disorder on chromosomes 14, 19, and 21. A minor early-onset FAD gene on chromosome 21 has been identified to encode the amyloid precursor protein (APP), and the late-onset FAD susceptibility locus on chromosome 19 has been shown to be in linkage disequilibrium with the E4 allele of the APOE gene. Meanwhile, the locus responsible for the major form of early-onset FAD on chromosome 14q24 has not yet been identified. By recombinational analysis, we have refined the maximal candidate region (MCR) containing the gene defect to approximately 3 megabases in 14q24. The candidate region lies between *D14S61* and *D14S289* based on obligate recombinant events observed with these markers in affected individuals from FAD kindreds individually exhibiting LOD scores > 3.0 with markers from the MCR. We will describe our laboratory's progress on attempts to finely localize this locus, as well as test known candidate genes from this region for either inclusion in the MCR, or the presence of pathogenic mutations. Candidate genes that have been tested so far include the oncogene *FOS*, heat shock protein 70 member (*HSPA2*), transforming growth factor beta 3 (*TGFB3*), the trifunctional protein C1-THF synthase (*MTHFD*), bradykinin B2 receptor (*BDKRB2*), and the E2k component of a-ketoglutarate dehydrogenase (*DLST*). *HSPA2*, *MTHFD*, and *BDKBR2* map outside of the current defined maximal candidate region, however, sequence analysis must be performed to confirm exclusion of these genes as true candidates. Meanwhile, no pathogenic mutations have yet been found in *FOS*, *TGFB3*, or *DLST*. We have also isolated a large number of novel transcribed sequences from the maximal candidate region in the form of "trapped exons" from cosmids identified by hybridization to select YAC clones and to oligonucleotides corresponding to known STS's in the region. We are currently in the process of searching for pathogenic mutations in these exons in affected individuals from FAD families, as well as isolating and characterizing cDNA clones obtained by screening with select exons.

AD and Prion Models

D3-010 THE AMINO TERMINAL FRAGMENT OF THE AMYLOID β-PEPTIDE MODULATES AMYLOID FORMATION.
Blas Frangione, Nibaldo C. Inestrosa, Eduardo M. Castaño, Claudio Soto-Jara. Department of Pathology, New York University Medical Center, New York, NY 10016

Amyloid β peptide (Aβ) consists of a hydrophobic C-terminal domain (residues 29-42), which adopts β-strand conformation, and an N-terminal domain (amino acids 10-24) whose sequence permits an α-helix and a β strand in dynamic equilibrium. We analyzed the effect of these different N-terminal conformations on amyloid fibril formation through the study of analogous Aβ peptides containing single amino acid substitutions. The single change of valine 18 for alanine induces a significant increment of the α-helical content of Aβ, determined by Fourier-transform infrared spectroscopy (FT-IR), and dramatically diminishes fibrillogenesis, measured by turbidity, thioflavine-T binding, Congo red staining and electron microscopy examination. In Hereditary Cerebral Hemorrhage with Amyloidosis Dutch type, a variant of Alzheimer's disease, the substitution of glutamine for glutamic acid at position 22 decreased the propensity of Aβ N-terminal domain to adopt an α-helical structure, with a concomitant increase in amyloid formation. We propose that Aβ exists in an equilibrium between two species: one an amyloidogenic conformation ($A\beta_{ac}$) and the other nonamyloidogenic ($A\beta_{nac}$), depending on the secondary structure of the N-terminal domain. Small perturbations of their equilibrium (caused by local pH changes, binding to pathological chaperones, etc.) could result in major changes in the capacity of Aβ to form amyloid fibrils. This is an alternative view to the current model, in which the C-terminal fragment plays the determining role for Aβ aggregation. Thus, the hypothesis proposed here opens new possibilities for the search for therapeutic agents that could promote the α-helix formation in the N-terminal domain of Aβ.

D3-011 MOLECULAR GENETIC AND BIOPHYSICAL STUDIES OF PRIONS CAUSING NEURODEGENERATION, Stanley B. Prusiner and Stephen J. DeArmond, University of California, San Francisco, CA 94143-0518

Prions are novel pathogens which are different from both viroids and viruses; this concept has received increasing support from many avenues of investigation over the past decade. Enriching fractions from Syrian hamster (SHa) brain for scrapie prion infectivity led to the discovery of the prion protein (PrP). Prion diseases of animals include scrapie and mad cow disease; those of humans present as inherited, sporadic and infectious neurodegenerative disorders. Although the formation of PrP^{Sc} from PrP^{C} is a post-translational process, no candidate chemical modification was identified, suggesting that a conformational change features in PrP^{Sc} synthesis. PrP^{C} contains ~42% α-helix and virtually no β-sheet; in contrast, PrP^{Sc} has ~30% α-helix and ~43% β-sheet. These data argue that the conversion of α-helices into β-sheets underlies the formation of PrP^{Sc}; however, initiation of this process by an undetected chemical modification in a small fraction of PrP^{Sc} remains a possibility. The fundamental features of prion structure and propagation differentiate prions from all other transmissible pathogens. The inherited human prion diseases are genetically linked to mutations in the PrP gene that result in non-conservative amino acid substitutions. Transgenic (Tg) mice expressing both SHa and mouse (Mo) PrP genes were used to demonstrate that the "species barrier" for scrapie prions resides in the primary structure of PrP. This concept was strengthened by the results of studies with mice expressing chimeric Mo/SHa transgenes from which "artificial" prions have been synthesized. Similar chimeric Mo/human (Hu) PrP transgenes were constructed which differ from MoPrP by 9 amino acids between residues 96 and 167. All of the Tg(MHu2M) mice developed neurologic disease ~200 days after inoculation with brain homogenates from three patients who died of Creutzfeldt-Jakob disease (CJD). Inoculation of Tg(MHu2M) mice with CJD prions produced $MHu2MPrP^{Sc}$; inoculation with Mo prions produced $MoPrP^{Sc}$. The patterns of $MHu2MPrP^{Sc}$ and $MoPrP^{Sc}$ accumulation in the brains of Tg(MHu2M) mice were different. About 10% of Tg(HuPrP) mice expressing HuPrP and non-Tg mice developed neurologic disease >500 days after inoculation with CJD prions. The different susceptibilities of Tg(HuPrP) and Tg(MHu2M) mice to human prions indicate that additional species specific factors such as chaperone proteins are involved in prion replication. Diagnosis, prevention and treatment of human prion diseases should be facilitated by Tg(MHu2M) mice. In other studies, Tg mice were compared expressing wt and mutant MoPrP. Overexpression of the wtMoPrP-A transgene ~8-fold was not deleterious to the mice but it did shorten scrapie incubation times from ~145 d to ~45 d after inoculation with murine scrapie prions. In contrast, overexpression at the same level of a MoPrP-A transgene mutated at codon 101 (corresponding to codon 102 in HuPrP) produced spontaneous, fatal neurodegeneration between 150 and 300 d of age in two lines of Tg(MoPrP-P101L) mice designated 2866 and 2247. Genetic crosses of Tg(MoPrP-P101L)2866 mice with gene targeted mice lacking both PrP alleles ($Prn\text{-}p^{0/0}$) produced animals with a highly synchronous onset of illness between 150 and 160 days of age. The $Tg(MoPrP\text{-}P101L)2866/Prn\text{-}p^{0/0}$ mice had numerous PrP plaques and widespread spongiform degeneration in contrast to the Tg2866 and 2247 mice that exhibited spongiform degeneration but only a few PrP amyloid plaques. Another line of mice designated Tg2862 overexpress the mutant transgene ~32-fold and develop fatal neurodegeneration between 200 and 400 d of age. Tg2862 mice exhibited the most severe spongiform degeneration and had numerous, large PrP amyloid plaques. While mutant $MoPrP^{C}(P101L)$ clearly produces neurodegeneration, $wtMoPrP^{C}$ profoundly modifies both the age of onset of illness and the neuropathology for a given level of transgene expression. These findings and those from other studies suggest that mutant and wtPrP interact, perhaps through a chaperone-like protein as noted above in studies of Tg(MHu2M) mice, to modify the pathogenesis of the dominantly inherited prion diseases. It seems likely that the lessons learned from studies of prion diseases may provide important insights into the etiologies and pathogenesis of the more common neurodegenerative diseases including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis.

Cellular and Molecular Biology of Alzheimer's Beta Amyloid Precursor Protein

D3-012 FUNCTION OF AMYLOID ßA4 PROTEIN AND ITS PRECURSOR APP IN HEALTH AND DISEASE, Konrad Beyreuther[1], and Colin L. Masters[2], [1]ZMBH, University of Heidelberg, D-69120 Heidelberg, Germany, [2]Department of Pathology, University of Melbourne, Parkville, Victoria 3052, Australia

The biogenesis of the amyloid protein precursor (APP), ßA4 protein and ßA4 amyloid has been studied by us both *in vitro* and *in vivo*. Synthetic ßA4 protein readily forms ß sheets, filaments and amyloid at micromolar concentrations. The principle to inhibit this process has been worked out by our groups with ßA4 variants. We also showed that at physiological concentrations (nanomolar) ßA4 aggregation requires amino acid oxidation and protein cross-linking by radical generation systems. The addition of radical scavangers such as ascorbic acid, tocopherol derivatives, and free amino acids prevent radical induced ßA4 aggregation. From necropsy examinations we estimate that a thirty-year period of ßA4 amyloid accumulation follows this initiation before clinical recognition. In cells that exhibit cell interaction, APP may exist in up to eight different isoform generated by alternative splicing of exons 7, 8 and 15. L-APP's, lacking exon 15, are abundant isoforms except in muscle and neurons. Neurons, the primarily affected cells in AD, are the only cells in brain that constitutively express APP. Splicing in neurons generates the four APP isoforms containing exon 15. Neurons do not express detectable levels of L-APP mRNAs. This is unique to neurons and may explain why AD is a CNS disorder. In contrast to neurons, only activated microglia and astrocytes highly express all eight APP isoforms. Interestingly, in microglia APP metabolism is regulated by cytokines and molecules of the ECM. In neurons, APP serves a synaptic function. Using immunoelectron microscopy APP was localized in neuronal perikarya, axons, dendrites and at pre- and postsynaptic densities. Newly synthesized APP is first delivered from the neuronal cell body to the axonal cell surface where it can be labeled with antibodies. Axonally labeled APP can move by transcytosis into dendrites and to the dendritic membrane which suggests that APP is the first neuronal molecule for which a transcellular pathway has been shown. Because transcytosis is a well known mechanism in epithelia to transport receptors and ligands either in the basolateral or in the apical direction, neuronal APP may be able to play a role in communicating between the pre- and post-synaptic environments of neurons. Molecules that can be monitored by APP include cell surface receptors and components of the extracellular matrix such as heparinsulfate proteoglycans, collagen and laminin to which APP binds. In this context it is interesting to note that APP has also non-overlapping binding sites for zinc(II) and copper(II). These metal ions regulate the homophilic dimeriziation of APP. Because dimer formation of receptors is one of the mechanism required for transmembrane signalling of non-pore forming transmembrane proteins, zinc(II) and copper(II) may be able to inhibit the APP-mediated communication between the pre- and post-synaptic environment. Zinc(II) and copper(II) are important cofactors in tissue-remodelling, wound-healing and electron transfer reactions and its binding protein APP appears to participate in these processes. The cerebral cortex and especially the hippocampus are exposed to high fluctuations of extracellular zinc(II) concentrations during synaptic transmission. The cholinergic deafferentation of the hippocampus is a neurochemical deficit in AD which increases the concentration of zinc(II) in this region. If APP is able to transmit these abnormal zinc(II) levels by a transcellular pathway from the pre- to the post-synaptic environment of these neurons, this could explain why the spread of the pathological changes in AD occurs along the neuronal projections.

D3-013 REGULATED CLEAVAGE OF β-AMYLOID PRECURSOR PROTEIN (APP): MOLECULAR & CELLULAR BASIS, Huaxi Xu, Robert Wallen, Mary Seeger, Paul Greengard & Sam Gandy, Department of Neurology & Neuroscience, Cornell University Medical College & Laboratory of Molecular & Cellular Neuroscience, Rockefeller University, New York

The relative utilization of alternative processing pathways for APP can be regulated by the activation state of certain protein phosphorylation signal transduction pathways. For example, activation of protein kinase C, or inactivation of protein phosphatases 1 and 2A, leads to a relative increase in utilization of the nonamyloidogenic, "α-secretase" cleavage pathway for APP processing.

The molecular and cellular basis for this regulatory event is one focus of our study. The possible mechanisms of regulated APP cleavage include (either singly or in combination) substrate (i.e., APP) activation or redistribution; or enzyme (i.e., α-secretase) activation or redistribution.

APP is a phosphoprotein; however, evidence from studies of the metabolism of mutant APP molecules indicate that changes in the APP cytoplasmic tail phosphorylation state may not be necessary for the phosphorylation-dependent activation of "α-secretase" cleavage, and indirect immunofluorescent studies of the subcellular distribution of APP in the absence or presence of phorbol esters (PKC activators) failed to disclose obvious phorbol-induced redistribution of APP immunoreactivity.

In order to elucidate the mechanism for regulated cleavage of APP, we have now reconstituted features of APP metabolism in *Saccharomyces cerevesiae* as well as in *in vitro* systems, such as porated, cytosol-deficient cells and Golgi-enriched subcellular fractions which generate nascent constitutive secretory vesicles. Current progress in using these systems for clarifying the molecular and cellular basis of regulated cleavage of APP will be discussed.

D3-014 REGULATION OF AMYLOID ß-PROTEIN SECRETION IN NORMAL CELLS AND IN FAMILIAL ALZHEIMER'S DISEASE, Dennis J. Selkoe, Martin Citron, Tsuneo Yamazaki, Marcia Podlisny, David B. Teplow and Christian Haass. Center for Neurologic Diseases, Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115

Progressive cerebral deposition of the amyloid ß-protein (Aß) is an invariant feature of Alzheimer's disease, preceding the clinical symptoms of the disease by many years. Aß is a proteolytic fragment of the ß-amyloid precursor protein (ßAPP), a type I integral membrane glycoprotein that is ubiquitously expressed in mammalian cells. Several missense mutations within or immediately flanking the Aß region of ßAPP have been strongly genetically linked to Alzheimer's disease in some families. We previously reported the constitutive secretion of Aß peptides from a variety of cells expressing ßAPP under normal culture conditions. These endogenously produced Aß peptides have heterogeneous N- and C-termini that vary as a function of the ßAPP missense mutations. Treatment of Aß-secreting cells with various agents that alter intravesicular pH showed that an acidic compartment is required for proper Aß generation. One such compartment appears to be the endosome. Immunolabeling of cell-surface ßAPP in living neurons and non-neuronal cells directly demonstrated the endocytosis of the protein and its rapid recycling (within 5-10 minutes) to the cell surface, as well as the trafficking of some internalized ßAPP molecules to late endosomes and lysosomes. Expression of truncated ßAPP molecules that have deletions of portions of the cytoplasmic domain, including the NPTY motif implicated in clathrin-mediated endocytosis, leads to decreased internalization and an associated decrease in the production of Aß peptides that begin at the usual aspartate$_1$ start site. These and other data suggest that Aß production begins with cleavage of the holoprotein by a still unknown protease(s) [designated β-secretase(s)] at the methionine-aspartate bond preceding the Aß N-terminus and that this cleavage can occur in early endosomes. To characterize the substrate requirements of the β-secretase, ßAPP was mutagenized by placing stop codons within or at the end of the transmembrane domain or substituting various amino acids for the wild-type methionine and aspartate at the P_1 and P_1' positions. These experiments showed that proper β-secretase cleavage of ßAPP to generate Aß requires the precursor to be membrane-anchored and is highly sequence specific, in that virtually all substitutions at the met or asp positions substantially decrease Aß production. Analogous mutagenesis experiments around the Aß C-terminus revealed that the unknown protease(s) cleaving here ["γ-secretase(s)"] does not show such peptide bond specificity.

In addition to their utility for characterizing the regulation of Aß production, cells constitutively secreting Aß might be useful for examining the critical issue of the aggregation of the Aß monomer into its neurotoxic polymeric form under physiological conditions. In this regard, we have found that CHO cells expressing normal or mutant ßAPP show aggregation of ≤ 10-20% of their secreted Aß peptides into SDS-stable dimers, trimers and sometimes tetramers under normal culture conditions. The occurrence of these small multimers has been confirmed by extensive immunochemical characterization and radiosequencing. They are present at ~100-500 pM levels in conditioned medium of CHO transfectants. Using this endogenous Aß aggregating system, we have begun to examine several variables that may influence aggregation and also compounds which may retard it.

In conclusion, studies of the regulation of Aß production and aggregation in cell culture can provide information under physiological conditions that can complement analyses of these processes *in vivo*.

D3-015 EXPRESSION AND METABOLISM OF APP, APLP1 and APLP2 IN VITRO AND IN VIVO, Sangram S. Sisodia[1,2], Gopal Thinakaran[1,2], Hilda H. Slunt[2], Amy C.Y. Lo[2,3], Cheryl A. Kitt[1,2], Cornelia von Koch[2,3], Song Wang[2,3], David R. Borchelt[1,2], Randall R. Reed[3,4], Hui Zheng[5], Donald L. Price[1,3,6]. Departments of [1]Pathology, [3]Neurology, and [4]Neuroscience, [2]Neuropathology Laboratory, [3]HHMI, The Johns Hopkins Univ. School of Medicine, Baltimore, MD., and [5]Merck Research Labs, Rahway, N.J.

Alzheimer's disease is pathologically characterized by the presence of amyloid plaques, composed primarily of Aß. Aß is derived from larger integral membrane glycoproteins, termed ß amyloid precursor proteins (APP). We have examined the metabolism of APP in cultured cells and demonstrate that soluble APP forms are secreted following endoproteolytic cleavage within the Aß region by a highly unusual cell surface protease, termed "α-secretase". We document that intracellular trafficking of APP and surface residence is largely influenced by sequences in the cytoplasmic tail. Our recent studies have focused on the metabolism and trafficking of APP harboring a mutation associated with early-onset AD, i.e., the Swedish variant. We document that in polarized epithelial cells (MDCK), APPSWE are cleaved at the "ß-secretase" site in an intracellular site and that the resulting soluble derivative ($APP^{sß}$) is secreted into the apical compartment with the vast majority of "α-secretase" generated APP^{s} being secreted basolaterally. Furthermore, we demonstrate that cleavage of APPSWE is initiated in the medial Golgi compartment and that "γ-secretase" acts at or near the cell surface to generate Aß. Parallel studies have focused on the expression and metabolism of APLP1 and APLP2, members of the APP gene family. We demonstrate that APLP2 is expressed at high levels in the nervous system and in peripheral tissues and that several APLP2 isoforms encoded by alternatively spliced transcripts are post-translationally modified by a chondroitin sulfate glycosaminoglycan (CS GAG) chain. Furthermore, CS GAG modification is regulated by insertion of sequences encoded by an alternatively spliced exon. Notably, expression of the CS GAG form of APLP2 appears restricted to embryonic neurons and mature neuronal populations which undergo regeneration, such as olfactory sensory neurons. Thus, differences in posttranslational modifications between the APLP2 isoforms are likely to underlie differences in the regulation and function of these isoforms. Our recent studies indicate that unlike APP and APLP2, APLP1 expression appears to be restricted to the nervous system during development and in mature animals. Our present efforts are directed towards using gene targeting strategies to disrupt expression of the mouse APP/APLP1/2 genes in order to define the normative roles of the encoded molecules in development and aging. Supported by the NIA, NINDS, American Health Assistance Foundation, Adler Foundation and a Zenith Award from the Alzheimer's Association.

Genetic and Biochemical Aspects of Huntington's Disease

D3-016 OXIDATIVE DAMAGE IN HUNTINGTON'S DISEASE, M. Flint Beal, Neurochemistry Department, Massachusetts General Hospital, Boston, MA 02114

Huntington's disease (HD) is a prototypical neurodegenerative disease characterized by selective neuronal depletion in the basal ganglia. Although the gene defect has recently been identified, the mechanism by which it contributes to neuronal degeneration remains obscure. We have hypothesized that a defect in oxidative phosphorylation may contribute to slow excitotoxic neuronal degeneration in this illness. We and others have obtained evidence for biochemical abnormalities in electron transport enzymes in Huntington's disease. Our most recent studies suggest that there may be an increase in complex I activity. We have also utilized magnetic resonance imaging spectroscopy to show that there are elevated lactate levels in both the basal ganglia and cerebral cortex of patients with Huntington's disease. We have carried out animal studies to determine whether it would be possible to mimic the neurodegenerative process with mitochondrial toxins. In particular we have focused on 3-nitropropionic acid, an irreversible inhibitor of succinate dehydrogenase. We have performed studies in both rodents and primates. These studies show that administration of this toxin systemically can result in lesions in the basal ganglia which show striking similarities to the neuropathologic and neurochemical features of Huntington's disease. At low dose levels there is sparing of NADPH-diaphorase neurons. In addition Golgi studies show that there are proliferative changes in the dendrites of spiny neurons which are similar to those which occur in Huntington's disease. These lesions also show age-dependence and are accompanied by impaired energy metabolism as documented with magnetic resonance spectroscopy in vivo. In primates one can produce an apomorphine inducible choreiform movement disorder which is reminiscent of that seen in Huntington's disease patients. The mechanism by which this toxin produces neuronal damage appears to involve both excitotoxic mechanisms and oxidative damage. We have been able to attenuate the lesions with both free radical spin traps as well as inhibitors of nitric oxide synthase. This suggests that peroxynitrite generation may be involved in the pathogenesis of these lesions. We have also examined whether agents which can improve energy metabolism might have efficacy in attenuating striatal lesions produced by the mitochondrial toxin malonate. We found that both coenzyme Q_{10} and nicotinamide can ameliorate lesions. Furthermore, they reduce lactate concentrations when administered to patients with HD. This raises the possibility that such an approach might prove useful in attempting to slow the neurodegenerative process.

D3-017 GENETICS AND PATHOPHYSIOLOGY OF HUNTINGTON'S DISEASE AND SMITH'S DISEASE, Christopher A. Ross, Laboratory of Molecular Neurobiology, Ross 618, Johns Hopkins Medical School, Baltimore, Maryland 21205-2196.

There are now known to be eight diseases caused by expanding triplet repeats, five of which are due to expansions of CAG repeats in long open reading frames. All of these are autosomal dominant disorders with age dependent penetrance involving degeneration of neurons in the central nervous system. The pathophysiology of these disorders is unknown, but has been suggested to involve excitotoxicity. For most CAG expansion diseases, the causative genes are widely expressed in both the brain and the periphery. Thus the puzzle in understanding their pathophysiology is how mutations in genes with wide expression lead to degeneration limited to selected populations of neurons. Huntington's disease (HD) will be taken as a paradigm for these disorders, with Smith's disease (or dentato-rubro and pallido-luysian atrophy, DRPLA) for comparison. We have generated antibodies to peptides in the predicted HD protein product. Like the mRNA, the protein is widely expressed in both the brain and the periphery and expression is not lost in patients with HD. Within neurons, it is present in the cytoplasmic compartment (or loosely associated with membranes) in cell bodies, dendrites and axons, and is enriched in a subpopulation of nerve terminals notably in the caudate and putamen. These findings are compatible with a possible role for the normal HD gene product in regulation of neurotransmitter release or reuptake, and perhaps with an involvement of the mutated protein product in excitotoxicity.

Trophic Factors As Potential Therapeutic Agents

D3-018 OVERVIEW OF MODELS OF CNS DEGENERATION AND INFLUENCE OF TROPHIC FACTORS, Franz Hefti, Ingrid Caras, Wei-Qiang Gao, Karoly Nikolics, Heidi S. Phillips, Cynthia Rask, Arnon Rosenthal, David Shelton, Gene L. Burton, and John W. Winslow, Genentech Inc., South San Francisco, CA 94080.

Clinical trials with neurotrophic factors are based on the anticipation that these molecules will reverse atrophy of the cell body's synthetic machinery and atrophic synaptic boutons, stimulate the synthesis of proteins necessary for transmitter release and, perhaps, reestablish lost synaptic contacts. Amyotrophic lateral sclerosis, which involves progressive degeneration of motor neurons of spinal cord, brain stem, and cerebral cortex, has become a preferred testing ground for neurotrophic factor therapy. Preclinical support for these trials was obtained in animals with acute experimental injury of motor neuron axons or mutant mice with developmental motor neuron atrophy. Ongoing or planned trials in peripheral sensory neuropathy are based on well established findings that most, if not all of the sensory neurons express one or several receptors of the Trk-family during adult life and on efficacy studies in animals with toxin-induced peripheral neuropathy. Survival of dopaminergic neurons, the cell population selectively affected in Parkinson's disease is promoted by a large number of growth factors in cell culture assays. Among the neurotrophins, BDNF and NT-4/5 are most effective, however, their effects in adult lesion models of dopaminergic neuron degeneration do not seem sufficient for the prediction that the neurotrophins will be able to attenuate dopaminergic cell degeneration in Parkinson's disease. GDNF, TGFβ2 and TGFβ3, are more potent and more effective than neurotrophins in promoting survival and differentiation of dopaminergic cells in culture and their expression pattern suggest a trophic role during development. GDNF has recently been found to protect adult rat dopaminergic neurons from atrophy or death induced by axonal transection. Ongoing clinical evaluation of NGF in Alzheimer's disease is based on the well established degeneration of cholinergic neurons in the disease and the robust trophic action of NGF on cholinergic neurons. A large body of evidence indicates that NGF prevents degenerative changes of cholinergic systems and associated behavioral deficits in adult animals. The conclusions have been confirmed recently by results obtained on mouse mutants lacking functional NGF or trkA genes. Analysis of neurotrophic factor actions on striatal neurons in culture, which are primarily affected in Huntington's disease, revealed that acute administration of NT-4/5 and BDNF increased survival of striatal neurons. These cell culture findings warrant further studies in animal models of this disease. Initial findings support the view that growth factors are involved in the response of the nervous system to acute injury as it occurs in stroke and traumatic injury. Death of cortical neurons in culture caused by glucose deprivation is attenuated by neurotrophins. In vivo, chronic intracerebral neurotrophic factor treatment in rats with experimental ischemia was reported to attenuate degenerative changes.

D3-019 FACTORS EFFECTING DIFFERENTIATION AND SURVIVAL OF MIDBRAIN DOPAMINERGIC NEURONS,
Arnon Rosenthal*, Heidi Philips*, Marc Tessier-Lavigne†, Kris Poulsen* and Mary Hynes*, *Department of Neuroscience, Genentech, Inc. 460 Point San Bruno Blvd., South San Francisco, CA 94080 and †Howard Hughes Medical Institute, Department of Anatomy, Programs in Cell Biology, Developmental Biology and Neuroscience, University of California, San Francisco, CA 94143-0452

Parkinson's disease is a progressive disorder caused by the degeneration of dopaminergic neurons in the midbrain. Potential future therapies for this disease include the intracranial administration of specific survival factors to slow the death of these neurons and/or transplantation of embryonic dopaminergic neurons as replacement therapy. We have identified two potent survival factors for dopaminergic neurons which prevent the death of cultured rat embryonic midbrain dopaminergic neurons at picomolar concentrations. These are TGF-β2 and β3, secreted factors which are distantly related to GDNF (a known survival factor for dopaminergic neurons). During embryogenesis, the distributions of these factors reveal that TGF-β2, β3 and GDNF are each sequentially expressed as local and target-derived trophic factors for dopaminergic neurons. Post-naturally and in adults, subpopulations of dopaminergic neurons, which project to distinct targets, have access to only one of the factors. Our findings provide evidence that GDNF, TGF-β2 and TGF-β3 are physiological survival factors for developing midbrain dopaminergic neurons and that each may be useful in preventing the degeneration of dopaminergic neurons in Parkinson's patients. Transplantation therapy requires large numbers of viable neurons for each patient, and we have initiated experiments to facilitate the production of large numbers of these neurons in vitro, for transplantation purposes. We find that the floor plate (a specialized group of cells occupying the ventral midline of the neural tube) is a cellular source of a factor that directs midbrain neuronal progenitors to assume a dopaminergic cell fate. In vitro, an exogenous floor plate placed in contact with embryonic midbrain tissue induces dopaminergic neurons to appear in tissue from which they normally derive as well as in midbrain tissue where these neurons normally never appear. Likewise, in transgenic mice in vivo, the production of a second floor plate at an ectopic dorsal site causes the animals to develop two sets of dopaminergic neurons, one in the normal location and one in an ectopic dorsal location. The molecular identification of this inducer may allow us to produce large numbers of dopaminergic neurons in culture, for eventual use in transplantation therapy for Parkinson's disease.

D3-020 CILIARY NEUROTROPHIC FACTOR (CNTF) BIOLOGY AND INFLUENCE ON MOTONEURONS, M. Sendtner[1,2], R.A. Hughes[2], F. Dittrich[1,2], B. Holtmann[2], Y. Masu[2], P. Carroll[2], G. Ochs[1], K.V. Toyka[1], H. Thoenen[2] [1]Department of Neurology, University of Würzburg, 97080 Würzburg and [2]Max-Planck-Institute for Psychiatry, Department of Neurochemistry, D-82153 Martinsried

The survival and functional maintenance of spinal motoneurons, both during the period of developmental cell death and in adulthood, have been shown to be dependent on trophic factors. In vitro experiments have previously been used to identify several survival factors for motoneurons, including CNTF and Leukemia inhibitory factor (LIF). Lesion of the facial nerve in newborn rats leads to the degeneration of more than 75% of the corresponding motoneuron cell bodies in the facial nucleus. Local application of CNTF and LIF can significantly prevent cell death of these motoneurons. The responsiveness of motoneurons to multiple factors *in vitro* and *in vivo* suggests that motoneuron survival and function are regulated by coordinated actions of these molecules as well as members of other gene families.

In order to study the individual physiological roles of neurotrophic factors on motoneurons, we have established transgenic mice where the genes for CNTF, LIF, and other neurotrophic factors have been disrupted. In CNTF deficient mice, progressive postnatal motoneuron degeneration can be observed, indicating that this factor is necessary for the maintenance of postnatal motoneuron survival, and that deficiency of CNTF in such mice cannot be fully compensated by other endogenous neurotrophic factors.

Intermittant pharmacological use of CNTF, as studied by daily subcutaneous injection of CNTF in *pmn* (progressive motoneuronopathy) mice, does not lead to significant functional improvement and rescue of degenerating motoneurons, whereas continuous supply of CNTF by intraperitoneal injection of CNTF secreting D3 cells markedly improved motoneuron survival and function. This could be due the the short halflife of CNTF in the circulation. In adult rats, intravenously injected radioiodinated CNTF is rapidly removed from the blood with an initial plasma halflife of 2.9 minutes. Most of the injected CNTF is bound to the liver. Liver cells express specific binding proteins for CNTF, and the incorporation and degradation of intravenously injected CNTF by the liver may occur after association of CNTF with the soluble CNTFR α in the circulation. Probably as a consequence of its binding to hepatocytes, CNTF induces acute-phase responses in liver. First results obtained from intrathecal administration of CNTF in sheep have shown that in the cerebrospinal fluid CNTF binds to a protein with properties similar to CNTFR α and leads to severe side reactions such as induction of CD-4 in microglial cells, lymphocytosis, and breakdown of the blood-brain barrier. Our results indicate that the potential therapeutic use of these factors in human motoneuron disease depends on the identification of optimal ways of administration and a better understanding of how these factors interact physiologically in regulating motoneuron function and survival.

Cellular Grafts and Delivery

D3-021 GRAFTING GENETICALLY MODIFIED CELLS TO THE BRAIN, F. H. Gage, The Salk Institute, La Jolla, CA 92093.

Intracerebral grafting has emerged as a useful experimental tool to address a variety of questions in neurobiology. Furthermore, the ability to restore function through neuronal grafting in the CNS has suggested a potential approach to CNS therapy through the selective replacement of cells lost as a result of disease or damage. Independently, the development of molecular genetic tools has begun to suggest genetic approaches to the treatment of human diseases. A combination of these two techniques, namely the intracerebral grafting of genetically modified cells, has been shown to be a useful tool to address biological issues, and an advanced approach toward the restoration of function in the damaged or diseased CNS. Neurotrophic factors are molecules that support the survival of selective neuronal processes during development. These molecules have also been shown to protect adult and aged neurons from undergoing degeneration. Furthermore, these factors have been shown to promote the growth of intact and damaged axons. When trophic factors are delivered to the brain via somatic cell transplantation, evidence for functional regeneration has now been established. The anatomical and physiological results of intracerebral grafting of cells genetically modified to secrete neurotrophic factors will be presented. As an extension of the effects of growth factors on neurons, evidence now exists that specific growth factors can support the survival and induce proliferation of fetal and adult stems that can be induced to differentiate into neurons and glia in vitro. Furthermore these stem cells can be genetically modified and subsequently grafted to the adult nervous systems where the terminally differentiate as either glia or neurons depending n the site into which they migrate. These recent findings are being used to design experiments to redefine our basic understanding of neuronal regeneration in the adult nervous system.

D3-022 PHYSIOLOGIC-BASED STRATEGIES FOR PEPTIDE DRUG DELIVERY TO THE BRAIN, William M. Pardridge, Department of Medicine, and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Recombinant neurotrophic factors or anti-Aß monoclonal antibodies are potential therapeutic and diagnostic agents in neurodegenerative diseases, such as Alzheimer's Disease (AD). However, such biotechnology products are not transported through the brain capillary endothelial wall, which makes up the blood-brain barrier (BBB) in vivo. A significant "bottleneck" in drug development for the therapy or diagnosis of neurodegenerative diseases is the requirement for effective strategies for protein drug delivery through the BBB. Two such strategies include chimeric peptides for the brain delivery of recombinant proteins and cationized antibodies for the brain delivery of monoclonal antibodies. Chimeric peptides are formed by conjugating a neurotrophic factor to a brain drug delivery vector. The latter is comprised of modified proteins or monoclonal antibodies that undergo absorptive-mediated or receptor-mediated transcytosis through the BBB and includes such proteins as cationized albumin or monoclonal antibodies that not only bind BBB surface antigens, but are transcytosed through the endothelial barrier. The conjugation of neurotrophic factors to brain drug delivery vectors is facilitated with the use of avidin-biotin technology, wherein a stable thiol-ether conjugate of the transport vector and neutral avidin is prepared in parallel with monobiotinylation of the recombinant protein. Previous studies have shown that in vivo CNS pharmacologic effects are obtained following systemic administration of chimeric peptides (PNAS, 90, 2618, 1993), and that brain drug delivery of a neurotrophic factor, brain derived neurotrophic factor (BDNF), is increased at least tenfold with this delivery technology (Pharm. Res., 11, 738, 1994).

Radiolabeled anti-Aß monoclonal antibodies are potential diagnostic neuroimaging agents for AD, should these molecules be made transportable through the BBB. One such strategy is the use of cationized monoclonal antibodies, whereby the isoelectric point (pI) is elevated by conversion of external carboxyl groups on the antibody into extended primary amino groups. Recent studies have described the characterization of cationized, radiolabeled anti-Aß monoclonal antibodies and the retention of binding of the modified antibody to the Aß amyloid in paraffin sections of autopsy AD brain (Bioconj. Chem., 5, 119, 1994) . An important element in the development of either cationic antibodies or chimeric peptides is the optimization of the plasma pharmacokinetics. The brain delivery of a chimeric peptide or cationic antibody is not only a function of the increase in BBB permeability caused by the delivery system, but also is directly related to the average plasma concentration. The plasma pharmacokinetics of radiolabeled, cationized anti-Aß monoclonal antibodies have been optimized and studies are ongoing to measure the retention of this agent by brain of living rodents and primates.

Late Abstract

PRIMATE MODELS OF PARKINSON'S DISEASE; GENETICALLY ENGINEERED CELLS IMPLANTATION APPROACH

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Description of non-human models of Parkinson's disease (PD) and demonstrating the ability of grafts of genetically modified cells to ameliorate behavioral symptoms in animal models of Parkinson's disease forms the central objectives of this presentation. While no animal model accurately recapitulates the slow progressive nature of PD, overlesioned hemiparkinsonian (HPD+)monkey model can mimic certain neuropathological, neurochemical and many functional deficits associated with Parkinson's disease.

17 Rhesus monkeys were studied during 9-18 months after selective dopaminergic neurotoxin (MPTP) lesion. The monkeys received an intracarotid infusion of 2-4 mg of MPTP supplemented by 0.4 mg/kg i.v. to induce HPD+, which still enabled them to sustain themselves. Apomorphine-induced turning, arm use, general activity measurements, clinical recordings using rating scale, in vivo microdialysis and CSF levels of HVA were examined. After the MPTP treatment, all of the monkeys showed progressively bilateral parkinsonian signs (freezing, bradykinesia, decreased defense reaction, posture and gait problems), and hemiparkinsonian signs with predominant use of the arm ipsilateral to the side of MPTP administration (rigidity, action tremor and decreased use of contralateral arm, ipsilateral spontaneous circling and apomorphine-induced contralateral turning).

7 HPD+ monkeys were used for study of intrastratal grafts of genetically modified autologous fibroblasts to produce and deliver L-DOPA. Using MRI guided stereotaxic surgery 3 monkeys were grafted with autologous fibroblast transfected with retroviral vector (MFGS) carring a human tyrosine hydroxylase (TH2) cDNA, 2 monkeys were implanted with non-transfected fibroblasts and 2 animals were left non-implanted. Prior to the implantation all animals were behavioraly characterized for 9-12 months using parkinsonian rating scale, arm use scale and activity monitors. TH implanted monkeys showed immediate and significant clinical improvement after the implantation which was followed for 4 months, significant return of parkinsonian arm function in all 3 animals at 2 months and in 1 monkey at 4 months. Animals were sacrificed at 4 months. There was no significant clinical improvement seen in the sham implanted or control monkeys. MRI scanning detected grafts in the striatum and closure of the blood brain barrier at 2 weeks post implantation. Histological examination of the implanted animals showed surviving fibroblast grafts, there were well vascularized and integrated in the host striatum. In all TH-fibroblasts-implanted monkeys TH mRNA was detected by *in situ* hybridization in the grafts, and in 1 animal TH-immunoreative fibroblasts were detected in the implant. Nither TH mRNA hybridization nor TH-immunoreactive staining were present in grafts in the sham implanted animals.

These results validate the potential application of ex-vivo gene therapy for neurodegenerative brain disorders.

Developmental and Experimental Models of Cell Death in the Nervous System; Influences of Trophic Factors in Cell Survival

D3-100 LIGANDS FOR THE EPH-RELATED KINASES (LERKs) ARE DEVELOPMENTALLY REGULATED IN THE BRAIN AND ARE EXCITO-PROTECTIVE FOR HIPPOCAMPAL NEURONS, Douglas Pat Cerretti, Carl Kozlosky, Nicole Nelson, Tim VandenBos, Fred Fletcher, Heather Shilling, Barry Davison, and Melissa K. Carpenter, Immunex Corporation, 51 University St. Seattle, WA 98101.

Hek and elk are members of the eph subfamily of receptor tyrosine kinases which are expressed in the developing and mature nervous system. We have previously described the isolation of four cDNAs that encode membrane bound proteins that are ligands for both hek and elk [Beckmann *et al.* (1994) *EMBO J.*, **13**:3757; Kozlosky *et al.* (1994) *Oncogene*, **9**:in press]. We have now isolated a fifth cDNA, termed LERK-5. LERK-5 is most similar to LERK-2 in that it is a type 1 transmembrane protein. In contrast, LERK-1, LERK-3, and LERK-4 are anchored to the membrane by GPI-linkage. LERK-5 has an overall amino acid identity of 59% with LERK-2 and has similar binding properties with LERK-2 in that LERK-5 is a ligand for both elk and hek with a higher affinity constant for the former. LERK-5 mRNA (~5 kb) was found primarily in fetal heart, lung, kidney, and brain as well as in the adult lung and kidney. LERK-2 is expressed in a variety of tissue and is developmentally regulated. We have also found that elk and LERK-2 are expressed in the developing hippocampus, cerebral cortex, the diencephalon, olfactory bulb, and the brain stem. Expression levels in all areas examined decrease with age. Expression of both mRNAs were also found in cultured hippocampal neurons, cortical neurons, and astrocytes. We also found that LERK-2 is able to protect cultured embryonic hippocampal neurons from glutamate excitotoxicity.

D3-101 CHANGES IN GLUTATHIONE METABOLISM SUGGEST A CRITICAL ROLE FOR THE REDOX EQUILIBRIUM IN NEURONAL DEATH AFTER TROPHIC FACTOR DEPRIVATION. Thomas L. Deckwerth and Eugene M. Johnson, Jr. Department of Molecular Biology and Pharmacology, Washington University School of Medicine, St. Louis, MO 63110.

Trophic factor-dependent sympathetic neurons die by apoptosis when undergoing programmed cell death after deprivation of their trophic factor, nerve growth factor (NGF) (Deckwerth and Johnson, *J. Cell Biol.* 123:1207-1222, 1993). Recent evidence suggests a potential role for oxidative stress in apoptotic death. To study the role of oxidative stress in the mechanism underlying death by deprivation of trophic factor, we analysed how trophic factor deprivation affects neuronal levels of the principal endogenous antioxidant glutathione and how experimental manipulation of glutathione levels affects viability.

Upon withdrawal of NGF from primary cultures of neonatal rat sympathetic neurons maintained by NGF, total neuronal glutathione increased transiently prior to any loss of viability. A similar increase could be produced by exposure of neuronal cultures to the oxidative stress-inducing agent 2,[3]-*tert*-butyl-4-hydroxyanisole and occurred *in vivo* in sympathetic ganglia during the period of developmental neuronal death. The transient increase is consistent with a neuronal antioxidant response to oxidative stress following the onset of trophic factor deprivation. Viability of NGF-maintained neurons depends acutely on the maintenance of adequate glutathione levels since depletion of glutathione by inhibition of its *de novo* synthesis triggered apoptosis with morphological and pharmacological characteristics similar to those of NGF deprivation-induced death. Death by NGF deprivation could be prevented by the antioxidant and glutathione-elevating agent N-acetyl L-cysteine, but only when given during the period of the antioxidant response. These results are consistent with the hypothesis that a disturbance of the cellular redox equilibrium caused by trophic factor deprivation can trigger the apoptotic death program. Supported by the Washington University Alzheimer's Disease Research Center (P50-AG05681).

D3-102 GENETIC ANALYSIS OF CELL DEATH IN CULTURED NEURONS, Henryk Dudek and Michael E. Greenberg, Division of Neuroscience, Department of Neurology, Children's Hospital, and Department of Neurobiology, Harvard Medical School, Boston, MA 02115

Considerable progress has recently been made towards identifying the proteins and mechanisms involved in cell death, particularly for apoptosis or programmed cell death. Some of these proteins, such as bcl-2, have been shown to be capable of regulating neuronal apoptosis. However, it is not yet certain whether other cell death mechanisms identified in non-neuronal cells, such as abnormal cell cycle regulation, also are involved in neuronal apoptosis. In addition, it is not known whether proteins which regulate neuronal apoptosis can also regulate other forms of neuronal cell death, such as excitotoxicity. To address these issues, we are testing the ability of a number of cellular regulatory proteins, such as cyclins and bcl-2 family members, to promote or inhibit neuronal cell death. To do this, we have developed a new method for transfection of DNA into primary neurons, which is based on calcium phosphate coprecipitation. This method is simple, non-toxic, and efficient; the transfected cells can be readily identified by immunostaining for a cotransfected marker protein, or by direct fluorescence upon transfection of the gene for Green Fluorescent Protein. We are using this transfection method to test the effect of specific genes on the viability of neurons and on their susceptibility to toxic treatments.

D3-103 PRION PROTEIN FRAGMENT 106-126 INDUCES INTRACELLULAR CALCIUM RISE THROUGH L-TYPE CALCIUM CHANNELS AND ASTROGLIAL PROLIFERATION. Tullio Florio, Maurizio Grimaldi, Antonella Scorziello, Mario Salmona°, Orso Bugiani*, Fabrizio Tagliavini*, Gianluigi Forloni° and Gennaro Schettini. Dipartimento di Neuroscienze e della Comunicazione Interumana, Università degli Studi di Napoli Federico II, via S. Pansini 5, I-80131 Napoli, °Istituto di Ricerche Farmacologiche Mario Negri, I-20157 Milano,*Istituto Nazionale Neurologico Carlo Besta, I-20123 Milano, Italy.

The prion protein (PrP) is one possible etiologic agent of transmissible neurodegenerative diseases of humans and animals such as Creutzfeldt-Jakob disease, and scrapie. These conditions are characterized by neuronal cell loss, vacuolation, amyloid deposition and astrocytes proliferation. PrP is expressed also in normal brains, but during the development of these conditions it is converted in a pathogenic form named PrP^{SC}, that is distinguishable from the native protein for its resistance to proteolysis and accumulation into the brain, generating amyloid fibrils. It was reported that the PrP fragment 106-126 is able to mimic most of the PrP^{SC} effects, causing amyloid deposition and apoptosis of neurons in culture. Here we report that the PrP106-126 fragment directly induce rat cortical type I astrocytes proliferation . This effect was not induced by a PrP106-126 scrambled peptide but was antagonized by the voltage-sensitive calcium channel blocker nicardipine and by the protein kinase C inhibitor staurosporine, Conversely, inhibitors of protein kinase A, NMDA receptor calciun channel or tyrosine phosphatases did not affect the PrP106-126-induced astrocytes proliferation. Moreover, microflurimetric studies showed that PrP106-126, but not PrP106-116 scrambled, caused a rapid increase in the cytosolic calcium concentration, completely reverted by the pretreatment with nicardipine.These findings demonstrate that the prion protein directly stimulates the proliferation of cortical astrocytes and that this effect may involve the increase in intracellular calcium concentrations through the activation of the L-type voltage-sensitive calcium channels. (Supported by CNR TP on Aging and MURST 40% to G.S.)

D3-104 A POSSIBLE ROLE OF TYROSINE HYDROXYLASE IN THE PATHOGENESIS OF PARKINSON'S DISEASE,
Jan Haavik, Bjørg Almås and Torgeir Flatmark, Department of Biochemistry and Molecular Biology, University of Bergen, N-5009 Bergen, Norway.
Several of the current hypotheses for the etiology of Parkinson's disease (PD) are based on the assumption that the loss of dopaminergic neurons in the *substantia nigra* is due to the formation of reactive oxygen species in the neurons, generated by nonenzymatic and/or enzymatic partial reduction of dioxygen. Multiple lines of evidence suggest that tyrosine hydroxylase (TH) may contribute to this oxidative stress: The distribution of the enzyme in the peripheral and central nervous system matches that of the neuronal degeneration found in PD, and the enzyme mechanism involves highly reactive activated oxygen species, which may break down to form oxygen free radicals during the "uncoupled" hydroxylase reaction. Recent studies on recombinant human TH isoforms, expressed in *E. coli*, have shown that a significant fraction of the tetrahydropterin consumption is not coupled to hydroxylation of substrate, when the enzyme is assayed under physiologically relevant conditions. Hydrogen peroxide was found to be formed in this partially uncoupled reaction. In comparison, a coupling efficiency of nearly 100 % was found for bovine adrenal TH, when the latter enzyme was assayed under identical conditions. Further studies on human TH expressed in alternative systems, including eukaryotic cells, are needed to clarify the physiological significance of these findings, including their possible relation to the progressive cell death found in PD.

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D3-105 SELECTIVE VULNERABILITY OF SUBPOPULATIONS OF DOPAMINE NEURONS TO DEGENERATION AND THE DIFFERENT LEVELS OF mRNA FOR THE DOPAMINE TRANSPORTER, D2 RECEPTOR, AND TYROSINE HYDROXYLASE IN MONKEYS. Suzanne Haber, Han Ryoo and Scott Evans. Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

There is a differential degeneration of the midbrain dopamine neurons in both Parkinson's disease and in MPTP-treated monkeys. The ventral tier subset of neurons is more vulnerable to cell death while the dorsal tier neurons is selectively spared. Several characteristics of dopamine cells have been associated with vulnerability to cell death including the dopamine transporter (DAT), and calcium binding proteins. Levels of these molecules and their message are not distributed uniformly in the subsets of dopamine neurons. The vulnerability subpopulations of dopamine neurons to cell death is likely to be related to a combination of biochemical characteristics of the cell. We found the levels of the DAT, D_2r, and TH mRNAs, and calbindin immunoreactivity is related to specific populations of cell loss after MPTP treatment in monkeys. Cells that have high levels of mRNA for the DAT and D_2r are more effected than cells that have relatively low levels. Furthermore, cells that show calbindin-positive ICC are less vulnerable to the effects of MPTP. These results are discussed with respect to the pathways associated with these cell groups.

D3-106 **AMPA-INDUCED TOXICITY IN RAT CORTICAL CULTURES IS ATTENUATED BY A SPECIFIC INHIBITOR OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE-II,** Iradj Hajimohammadreza, Peter A. Boxer and Kevin K.W. Wang. Department of Neurological & Neurodegenerative Disorders, Parke-Davis Research, Warner-Lambert Company, 2800 Plymouth Road, Ann Arbor, MI 48105.
Excess presence of glutamate in brain can induce severe neuronal death (eg. in ischemia). One major ionotropic glutamate receptor subtype is the AMPA receptor, which upon activation allows the influx of sodium and calcium ions, either directly or via depolarization. AMPA receptor antagonists have been used successfully in providing neuroprotection in *in vivo* models of ischemia. Recent studies have shown that AMPA currents are enhanced by calcium/calmodulin-dependent protein kinase-II (CamK-II). Thus we investigated the possibility that, specific inhibition of CamK-II would be neuroprotective against AMPA toxicity. We used KN-62, a specific and cell permeable inhibitor of CamK-II in rat fetal cerebrocortical cultures challenged with AMPA. Pre-treatment of cultures with KN-62 (5μM) provided a significant neuroprotection against toxic concentrations of AMPA, as seen with light microscopy, LDH assay and spectrin breakdown immunoblots. Furthermore, KN-62 significantly reduced AMPA-induced total calcium accumulation ($^{45}Ca^{2+}$), and elevation in $[Ca^{2+}]_i$ (Fura-2 AM). Our data suggest that CamK-II has a significant role in glutamate mediated neurotoxicity.

D3-107 THE ROLE OF LEUKEMIA INHIBITORY FACTOR RECEPTOR B (LIFRB) IN MURINE NERVOUS SYSTEM DEVELOPMENT, Simon A. Koblar*, Cynthia R. Willis#, Perry F. Bartlett*,and Carol B. Ware#.*Neuroimmunology Laboratory, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia 3050. #Immunex, Seattle, Washington 98101.
LIFRB is a common receptor subunit for the cytokines: leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), and oncostatin M (OSM). LIF stimulates sensory neuron development (M. Murphy et al., Development:1993; 117, 1173-82), and potentiates motor and sensory neuron survival (S. Cheema et al., J. Neurosci. Res.:1994; 37, 213-8). CNTF protects motor neurons from degeneration (M. Sendtner et al., Nature:1990; 345, 440-1), and when deficient, in a mouse null mutant, results in minor motor neuron loss postnatally (Y. Masu et al., Nature:1993; 365, 27-32). To investigate the role of LIFRB in development a murine homozygous null mutant has been generated. The mutant mice die perinatally. The most striking neural abnormality is a loss of glial fibrilliary acidic protein reactivity (GFAP) in the hindbrain and spinal cord at embryonic day (E) 17.5, suggesting a defect in astrocyte development. Otherwise, the remainder of the central and peripheral nervous systems appear normal on initial histological examination. Sensory neurons, dissected from perinatal mutant pups, die when cultured for two days in either LIF, CNTF, or OSM. This indicates that with the loss of LIFRB these cytokines are unable to signal effectively to keep sensory neurons alive in vitro. In contrast, nerve growth factor is still able to keep these neurons alive. Neural crest cultures from E9.5 mutant embryos were treated with LIF, and a severe defect in sensory neuron differentiation was seen compared to heterozygous and wild type cultures. Nevertheless, the neural crest was normal in migration and proliferation.
This data suggests that the LIFRB has an important role in glial and neuronal development. It will be interesting to determine further defects in neural development caused by the loss of the LIFRB.

D3-108 SEROTONIN-ACTIVATED ALPHA-2-MACROGLOBULIN (α2M) AND α1M RESPECTIVELY BLOCKS AND STIMULATES NEURITE OUTGROWTH OF RAT EMBRYONIC FOREBRAIN NEURONS AND ADULT RAT SCIATIC NERVE REGENERATION. Peter H. Koo and Daniel J. Liebl, Department of Microbiology and Immunology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Alpha-2-macroglobulin (α2M) is a major serum glycoprotein in all the vertebrates investigated. It also ubiquitously occurs in various body fluids and tissues, and is secreted by astrocytes and neurons. In rats two isoproteins (α1M and α2M) exist, and both can readily react with monoamines such as serotonin (5HT) to yield the activated molecules with unique biological activities. In our previous studies we have shown that monoamine-activated human α2M inhibits neurite outgrowth, survival, and choline acetyltransferase (ChAT) of embryonic neurons *in vitro*, and dopamine concentration of adult rat caudate putamen *in vivo*. In this study we investigated the effect of rat α1M and α2M on rat CNS and PNS neurons, as a model of study for neuroregulation and neurodegeneration. Rat 5HT-α2M can dose-dependently inhibit neurite-outgrowth and survival of embryonic rat basal forebrain neurons. But it is nontoxic to neurons and its inhibitory activity can be overcome by higher neurotrophin concentrations. Both 5HT-α2M and 5HT-α1M also decrease ChAT activity of embryonic basal forebrain neurons, but 5HT-α1M can dose-dependently stimulate neurite-outgrowth of these neurons *in vitro*. Rat 5HT-α2M again effectively blocks the regeneration of transected rat sciatic nerve *in vivo* in an interposed silicon chamber; 5HT-α1M, on the other hand, greatly stimulates sciatic nerve regeneration. Anti-α2M and anti-α1M antibodies applied to the implanted chambers respectively promote and block sciatic nerve regeneration. Hence these αM homologues may be natural physiologic regulators important for embryonic nerve development and adult nerve regeneration, and may play a significant role in the neurodegenerative diseases. (NIH NS-30698)

D3-109 PREMATURE NEUROIMMUNODEGENERATION (NID) - TWO MODEL SYSTEMS OF HYPERSENSITIVE APOPTOSIS, Lynn, W.S. and Wong, P.K.Y., Department of Carcinogenesis, University of Texas, M.D. Anderson Cancer Center, Science Park-Research Division, Smithville, TX 78957.

In the radiation-sensitive syndrome, Ataxia Telangiectasia, T cells and neurons slowly degenerate, without inflammation, followed by neoplasia. Cell cycling, lectin responsiveness, and growth factor production become impaired. Expression of factors controlling apoptosis, p53, and probably bcl-2, and IL-6 is altered and with redox stress or serum diprivation, the isolated T cells rapidly shrink with nuclear dissolution and cell death. This oxidative apoptosis cell death is rapidly controlled in vitro by appropriate use of N-acetyl cysteine.

Similarly, infection of neonatal FVB mice with the MuLV retrovirus, *ts*1, results in progressive disappearance of T cells and motor neurons with accumulation of cell growth modulators, e.g., TNF and IL-1. No acute inflammatory response is seen, but splenic hyperplasia with subsequent neoplasia and gliosis does occur. Splenocytes and thymocytes obtained from these *ts*1 infected mice are also very susceptible to serum deprivation or oxidant stress which is also controlled by N-acetyl cysteine. Symptomatic control of this neonatal murine model of NID is possible using either immune superantigens, such as polyinosine cytosine; antioxidants, e.g., acetyl cysteine and melatonin; cytokines, e.g., interferon γ and IL-2, or NMDA receptor analogues, but not by nitric oxide analogues. Evaluation of such therapies in vivo in the human models of NID, including HIV infection, may be useful.

D3-110 ALTERNATE- DAY DITARY RESTRICTION MEDIATED CHANGES IN GLUCOSE,PYRUVATE AND LACTATE PROFILES IN AGEING BRAIN OF MALE BALB/C MICE.

M.A.S.RAO and K.Shankaraiah, Dept.of.Zoology, Osmania University, Hyderabad-500 004, India.

It is well documented in detail that, dietary restriction alters the ageing process resulting in increased life span. The present investigation reports the changes in glucose,pyruvate and lactate profiles in different regions of brain of male BALB/C mice under the influence of alternate day dietary restriction. These metabolites were estimated in control (ad-lib fed) on-day experimental (fed on previous day) and off-day experimental (fasted on previous day) conditions. Glocose content was more in on-day experimental group in 3 and 6 month old animals. At 12 months of age more glucose content was observed in control group,while in 18 months old animals more amount of glucose was noticed in both experimetal groups than control. All brain regions exhibitted similar age related trend in pyruvate profiles in all regions of brain. Pyruvate content was more in off-day experimental group at the age of 18 months when compared to other groups. Where as, pyruvate content was decreased in all groups at the age of 12 months. Lactate content was observed to be age dependent and region specific. On-day experimental group exibitted more lactate over control and off-day experimental groups throughout the ageing. These results clearly indicates that,there is an overall shift in the energy metabolic path way in the event of dietary restriction. Indepth discussion was made regarding ageing and dietary restriction.

D3-111 NEUROTROPHIN RECEPTOR ACTIVATION CONTROLS DEVELOPMENT AND REGENERATION OF OLFACTORY NEURONS A. Jane I. Roskams, M. Angelyn Bethel, Laurie C. Williams and Gabriele V. Ronnett. Departments of Neuroscience and Neurology, Johns Hopkins School of Medicine, 725 N. Wolfe Street, Baltimore MD 21205.

Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins -3,4 and 5 (NT-3,4,5) have been identified and characterized by their ability to induce neuronal differentiation and support neuronal survival in vivo and in vitro. The interaction of these factors with the Trk family of receptor tyrosine kinases is complex and has been studied extensively in cell lines and ectopic expression systems. Neurotrophin receptor activation involves neurotrophins acting either individually through one Trk, through a complex of Trks or through a Trk complexed with the low affinity NGF receptor (LNGFR). We have detected expression of Trk A,B,C and the lNGR in olfactory neuroepithelium. The olfactory neuronal epithelium is unique in its ability to undergo neuronal regeneration throughout the lifetime of the organism. As such, neurons at different stages of maturation are found throughout the olfactory epithelium in the adult rat. Different subsets of neurotrophin receptors are expressed following unilateral bulbectomy within regenerating olfactory neuroepithelium in neurons at different stages of differentiation. In primary cultures of olfactory neurons Trks become specifically phosphorylated when challenged with the appropriate neurotrophin ligand and activate a signal transduction cascade that terminates in the up-regulation of immediate early genes such as fos and jun. We are currently investigating whether different individual neurotrophins utilize the same or different signal transduction pathways to achieve transcriptional activation during neurogenesis in olfactory neurons in vivo and in vitro.

D3-112 EXPRESSION OF HIGH AFFINITY NEUROTROPHIN RECEPTORS (TRK A, B, AND C) IN THE NUCLEUS BASALIS OF MEYNERT OF HUMAN BRAIN, A. Salehi*, J. Verhaagen*, D. Kaplan**and D. F. Swaab*.
*Graduate School Neurosciences Amsterdam, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands,**Eukaryotic Signal Transduction, Frederick Cancer Research and Development Center, P.O. Box B, Frederick, MD 21702-1201, U.S.A.

Recently it has been shown that a family of tyrosine kinases, the trks, function as high affinity receptors for neurotrophins in mammals. These receptors are designated trk A, B and C. NGF binds to trkA while trk B and C transduce biological responses of BDNF and NT-3. Studies of trk knock out mice and overexpression of trkA in cell lines has demonstrated that these receptors play an important role in neuronal differentiation and survival, while their involvement in neurodegeneration is also presumed. Currently no information on the cellular distribution of the trks in the human nervous system and possible changes in trk expression in neurodegenerative diseases is available. We studied the cellular distribution of the high affinity receptors for neurotrophins in the nucleus basalis of Meynert (NBM), an area of the brain which exhibits a strong degeneration and atrophy in neurodegenerative diseases like Alzheimer's disease. In order to localize these receptors in the human brain, we used three polyclonal antibodies directed against the external domain of the trk A, B and C. These antibodies are specific for trk A, B and C as has been shown previously by Western blot . We selected four control subjects to study the expression of trk receptors in the NBM. The blocks of brains containing the NBM fixed in paraformaldehyde and embedded in paraffin were stained immuncytochemically. All antibodies stained numerous magnocellular neurons in the NBM. Interestingly the expression of these receptors was not uniform at the level of the NBM. The highest number of immunoreactive neurons was observed for trk B which was followed by C and A. The immunoreactivity for trk receptors was observed throughout the neurons, including their cell bodies and projections. TrkA is predominantly expressed in basal forebrain including the vertical and horizontal bands of Broca and the NBM. However, trk B and C were expressed more widely, also in adjoining areas. This is the first study comparing the expression of all three trk receptors in the human NBM. Currently we are studying any possible alterations in the expression of these receptors in the NBM in AD.(Brain material was obtained from the Netherlands Brain Bank (coordinator R. Ravid).

D3-113 REGULATION OF GFAP GENE EXPRESSION IN RETINAL DEGENERATIONS, Vijay Sarthy and Lisa Verderber, Department of Ophthalmology, Northwestern University Medical School, Chicago, IL 60611.

Neuronal degeneration is usually accompanied by reactive gliosis and increased expression of the Glial Fibrillary Acidic Protein (GFAP) gene in neighboring astrocytes. In the mammalian retina, the GFAP gene is not normally expressed in Müller (glial) cells. The GFAP gene is, however, strongly activated in response to photoreceptor degeneration resulting from mutations, light damage or ischemia. Our research is concerned with identifying the molecular mechanisms responsible for GFAP gene activation, and in characterizing the extracellular signal mediating GFAP induction in Müller cells. Recent cell transfection assays show that regulatory elements needed for cell-specific, high level GFAP expression are present within 2 kb of 5' flanking sequence of the mouse GFAP gene. The sequences are, however, unable to stimulate GFAP expression in Müller cell cultures. Similarly, in GFAP-lacZ transgenic mice carrying 5' and 3' immediate, flanking sequences, β-gal is expressed in retinal astrocytes but not in Müller cells. These results indicate that GFAP expression in Müller cells is regulated by a cell type-specific, distal enhancer. Furthermore kinetic studies show that GFAP is found in Müller cells long after its initial synthesis probably as a consequence of its low turnover in the Müller cell cytoskeleton while GFAP mRNA levels rise and decline rapidly due to transient induction of the GFAP gene. Growth factors such as bFGF, CNTF, BDNF and IL-1 have been suggested as the extracellular inductive signaling molecules involved in GFAP induction. The cellular origin of these molecules and their site of action in the retina are being investigated currently. Supported by EY-03523.

D3-114 INVOLVEMENT OF INTRACELLULAR CALCIUM AND RNA-SYNTHESIS IN THE APOPTOSIS OF CEREBELLAR GRANULE CELLS: RESCUING BY HIGH KCl, FORSKOLIN AND IGF-1. Gennaro Schettini, Olimpia Meucci, Antonella Scorziello, *Thomas Werge, *Pietro Calissano and *Cinzia Galli, Dipartimento di Neuroscienze Sezione di Farmacologia Universita' di Napoli Federico II, 80131 Napoli, *Instituto di Neurobiologia, Consiglio Nazionale delle Ricerche, 00137 Roma, Italy.

Cerebellar granule cells deprived of depolarizing concentration of extracellular potassium, $[K^+]_o$, undergo apoptosis. We report that this apoptotic process is associated with an immediate and permanent decrese in the levels of free intracellular calcium $[Ca^{2+}]_i$. Althought forskolin and IGF-1 are both able to prevent apoptosis, only forskolin is able to counteract the instantaneous decrease of $[Ca^{2+}]_i$. However, the early effect of forskolin on $[Ca^{2+}]_i$ is lost after longer incubation in low $[K^+]_o$. The calcium antagonist nifedipine is able to interfere with the survival effect of high $[K^+]_o$, while it does not affect forskolin and IGF-1 promoted survival, as assessed by viability and genomic DNA analysis. Accordingly, the L-type calcium channels agonist Bay K 8644 significantly enhanced the survival of low KCl treated neurons.

We determined also the time course of the rescue capacity of high $[K^+]_o$, forskolin, IGF-1 and actinomycin D, in order to characterize the signal trasduction events and the essential trascriptional step in cerebellar granule cells apoaptosis. Addition of high KCl, forskolin or IGF-1, 6 hr after the initial KCl deprivation saves 50% of cells. Remarkably, 50% of neurons loss the potential to be rescued by actinomycin D after only 1 hr in low [K+]o. Finally we show that the survival promoting activities of high $[K^+]_o$, forskolin and IGF-1 do not require RNA synthesis.We conclude that $[Ca^{2+}]_i$ is involved in the survival promoting activity exerted by high $[K^+]_o$ but not in those of forskolin and IGF-1, and that all three agents, although rescuing neurons from apoptosis through distinct mechanisms of action, do not necessitate RNA trascription. (CNR T.P. on Aging 1994 to G.S.)

D3-115 NOVEL GENES INDUCED IN THE HIPPOCAMPAL FORMATION BY GLUTAMATE ANALOGS AND/OR NEUROTROPHINS, Lars Eyde Theill#, Greg Naeve#, Masayo Kornuc#, Rainer Kramer#, Elly Nedivi*, Dana Hevroni*, Amir Rattner*, and Yoav Citri*, #Dept. Cell Biology, Amgen Inc., Thousand Oaks, CA 91320, *Dept. of Hormone Research, The Weizmann Institute of Science, Rehovot, Israel.

Neural plasticity is exhibited during CNS development, in the adult brain during learning and memory processes and after epileptic seizures. Plasticity refers to changes in the strength and pattern of synaptic connections in response to neural activity. Transcription and protein synthesis is required for long-term persistence of plastic changes. We have set out to clone and characterize a large number of candidate plasticity-related genes (CPGs), study their expression pattern and for select genes investigate their function. A "clone by clone" cDNA cloning and screening strategy is used that allows for detection of very low abundance transcripts that become induced during a regiment that elicits strong neural activity (E. Nedivi, D. Hevroni, D. Naot, D. Israeli and Y. Citri (1993) Nature 363 718-722). Using this approach 170 cDNA's induced in the dentate gyrus by the glutamate analog kainate have so far been isolated and partially characterized. About 50 of the cDNAs represents known genes some of which encode neurotrophic factors, neuropeptides and vesicular and other synaptic components. About 120 clones appear novel and 30 of these have been selected for full-length cloning and sequencing. As determined by in situ hybridization several of the genes are also induced during natural CNS developmental processes. Brain derived neurotrophic factor (BDNF) is a strongly induced "immediate-early" gene following kainate treatment and we are therefore also investigating whether any of the CPGs are "target" genes for neurotrophins. We present here evidence that in vivo cerebroventricular administration of BDNF markedly induces some of the novel CPGs in the hippocampal formation as well as in the neocortex. The same genes: which includes a novel dual-specificity phosphatase; a new member of the kinase super-family and two novel genes encoding proteins with consensus signal peptides are also induced by BDNF and NT3 in primary hippocampal and/or cortical cultures. The function of these and other kainate induced genes such as a novel seven transmembrane domain receptor is under investigation.

D3-116 MAITOTOXIN INDUCES CALPAIN ACTIVATION IN SK-SY5Y NEUROBLASTOMA CELLS AND FETAL CEREBROCORTICAL CULTURES, Kevin K.W. Wang, Iradj Hajimohammadreza, Kadee J. Raser and Rathna Nath, Department of Neuroscience Therapeutics, Parke-Davis Pharmaceutical Research, Warner-Lambert Co., 2800 Plymouth Road, Ann Arbor, MI 48105

Maitotoxin is a highly potent marine toxin. It appears to open both voltage-sensitive and receptor-operated calcium channels in the plasma membrane. This results in calcium overload that rapidly leads to cell death. We now report that maitotoxin (1 nM) induces calpain activation in both SH-SY5Y neuroblastoma cells and fetal rat cerebrocortical cultures. Calpain activation was measured by both the formation of spectrin breakdown products (150 kDa, 145 kDa) and *in situ* hydrolysis of peptide substrate succinyl-Leu-Leu-Val-Tyr-7-amido-4-methylcoumarin. Removal of extracellular calcium prevented maitotoxin-induced calcium accumulation and calpain activation while receptor-operated calcium channel blocker SKF96365 also offered partial blockade. Calpain inhibitors Z-Val-Phe-H and PD150606 inhibited calpain activation in maitotoxin-treated SY5Y cells. Maitotoxin was much more effective than the calcium ionophore A23187 in activating calpain in SY5Y cells. Our results suggest that i) calpain overactivation may contribute to the toxicity of maitotoxin; ii) when used in modest concentrations, maitotoxin may be useful in investigating the physiologic and pathophysiologic roles of calpain in neuronal cells.

D3-117 NEURONAL CELL DEATH INDUCED BY MUTANT AMYLOID PRECURSOR PROTEIN VIA APOPTOSIS, Boyu Zhao[1], Sangram S. Sisodia[2], and John W. Kusiak[1]. Molecular Neurobiology Unit, Gerontology Research Center/NIA/NIH, Baltimore, MD 21224[1]; Department of Pathology, Johns Hopkins University, Baltimore, MD 21205[2].

The identification of several mutations in the amyloid precursor protein (APP) gene of certain familial Alzheimer disease pedigrees suggests that APP metabolism underlies the disease mechanism of these individuals. We previously demonstrated that NGF differentiated PC12 cells expressing mutant APPs (APP692, APP693 and APP717F) undergo morphological changes leading to cell death, which is correlated with an increased level of large carboxyl terminal fragments of APP within the cells (Neurobiology of Aging, 15(S1): S56, 1994). We now report evidence of the apoptotic nature of neurodegeneration in these cell lines. DNA from PC12 cells transfected with APP constructs exhibited a ladder of oligonucleosome-length fragments characteristic of apoptosis after exposure to NGF and cAMP for 6 days. The intensity of DNA ladders was increased in all mutant APP transfected cell lines as compared to wild-type APP transfected cells. These DNA fragmentation results in both wild-type and mutant APP transfected cell lines were confirmed by TUNEL *in situ* and flow cytometric techniques. The percentage of apoptotic cells was less than 0.1% in untransfected and vector transfected cells, 0.8% in wild-type APP transfected cells, and increased significantly in mutant APP transfected cell lines, up to 22%. Scanning electron microscopic analysis showed extensive soma blebbing in the cells expressing mutant APPs, resulting in a reduction of cell size. Transmission electron microscopic analysis revealed patches of condensed chromatin lying against the nuclear membrane and well-preserved mitochondria and Golgi organelle structures.

These results suggest that mutant APP induced neuronal degeneration occurs via an apoptotic mechanism, which may contribute to the neuronal loss in Alzheimer disease. Our *in vitro* culture system is useful to further study the cellular mechanisms of neurodegeneration induced by mutations of APP and to investigate rational therapeutic strategies.

Clinical and Genetic Aspects of Motor Neuron Disease; Models of Motor Neuron Disease

D3-200 THE ROLE OF MATURE SKELETAL MUSCLE FIBERS AND MUSCLE DERIVED NT4/5 FOR SURVIVAL OF MOTOR NEURONS DURING EMBRYONIC DEVELOPMENT IN TRANSGENIC MICE. Thomas J. Brennan and John W. Winslow, Department of Neuroscience, Genentech Inc. S. San Francisco, CA 94080

During both avian and mammalian embryonic development there is an approximately 50% loss of motor neurons due to naturally occurring cell death. We have generated transgenic mouse models to determine what factors are involved in this early selection process. Among the theories to explain naturally occurring motor neuron cell death are the motor neuron trophic theory and the target dependence theory. The motor neuron trophic theory suggests that a limited amount of a survival factor is produced in the vicinity of the neurons and only those motor neurons which receive enough will survive. The target dependence theory implies that some aspect of the muscle target, like myofiber size or number, synaptic sites, or other muscle specific features control motor neuron survival. In order to test the trophic factor theory we have created transgenic mice which overexpress NT4/5, a potent motor neuron trophic factor, in muscle. If motor neurons are dependent on NT4/5 or a related factor during development we would expect an increase in survival of motor neurons. To test the role of mature skeletal muscle fibers we are utilizing mice in which the muscle inducing gene, myogenin, has been deleted. These mice do not develop skeletal muscle fibers. If skeletal muscle target is involved in the selection process then we would expect to see a decrease in the number of surviving motor neurons.

D3-201 FUNCTIONAL CHARACTERIZATION OF THE BRAIN-SPECIFIC FGF-1 PROMOTER, FGF-1.B. Ing-Ming Chiu, René L. Myers, and Subir K. Ray, Department of Internal Medicine and Comprehensive Cancer Center, The Ohio State University, Columbus, OH 43210
We have previously shown that the expression of alternatively spliced human acidic fibroblast growth factor (aFGF or FGF-1) transcripts is regulated in a tissue/cell line specific manner via multiple promoters. The brain specific promoter, FGF-1.B, is a non-TATA promoter and contains multiple transcription start sites. In order to identify the cis-regulatory elements in the FGF-1.B promoter, we constructed a series of promoter deletions fused to the luciferase reporter gene. The transcriptional activity of these recombinants was determined following transfection into an FGF-1.B positive glioblastoma cell line, U1240MG and a 1.B negative cell line, U1242MG. Results of transient transfections indicate a few elements which are involved in the positive regulation of FGF-1.B expression. The core promoter is located in a 40 bp region, showing activity in U1240MG but not in U1242MG cells. Two positive elements are located within the 540 bp region 5' to the major transcription start site and are designated regulatory region-1 (RR-1) and RR-2. Electrophoretic mobility shift assays (EMSA) using radiolabeled probes for the 1.B promoter region have identified sequence specific binding sites in RR-1 and RR-2. Footprinting analysis by methylation interference of the positive regulatory element RR-2 identified three guanine residues which are important for protein binding. Mutants of RR-2 abolished binding to nuclear proteins and showed diminished luciferase reporter activity. These results confirmed RR-2 as a transcriptional enhancer element. The effects seen are specific for the U1240MG cell line supporting a role for RR-2 in the tissue specific regulation of FGF-1.B. Southwesertn analysis using an oligonucleotide probe derived from RR-2 (nucleotides -489 to -467) identified a 37-kD protein which is present in nuclear extracts from U1240MG and brain but not from U1242MG. Studies are ongoing to further characterize these and other interactions and to ascertain the mechanism of transcriptional activation of the brain specific FGF-1.B transcript.

D3-202 THE CYCAD TOXIN MAM ENHANCES NEURONAL TAU mRNA EXPRESSION PRODUCED BY GLUTAMATE, [1]F. Esclaire, [2]G.E. Kisby, [1]P. Sindou, [1]M. Lesort, [2]C. Sweatt, [2]P.S. Spencer, [1]J. Hugon. [1]Cellular Neurobiology Unit, Histology Laboratory, Faculty of Medicine, University of Limoges, 87025 Limoges, France; [2]Center for Research on Occupational and Environmental Toxicology, Oregon Health Sciences University, Portland, OR 97201.
Alzheimer's disease and Guam Amyotrophic Lateral Sclerosis and Parkinsonism-Dementia Complex (ALS/PDC) are neuropathologically characterized by the presence of neurofibrillary tangles in degenerating neurons. The major biochemical components of paired helical filaments are abnormally phosphorylated forms of the microtubule-associated protein tau. We have shown previously (a) that glutamate increases neuronal tau mRNA expression and tau phosphorylation, and (b) Guam ALS-PDC is associated with prior dietary and medicinal use of the seed of the neurotoxic cycad plant (*Cycas* spp.). The present study determines whether methylazoxymethanol (MAM), the genotoxic aglycone of the cycad glucopyranoside cycasin, augments the effects of glutamate on neuronal tau mRNA expression. Rat primary cortical neuronal cultures (8 days *in vitro*) were treated with MAM (10 μM) for 12 h, washed and held for 24 h in control culture medium, exposed to glutamate (50 μM) for 8 min, and maintained in culture medium for 12 h before analysis. Parallel groups of cultures were treated with MAM alone (10 μM), glutamate alone (50 μM), or neither compound (controls). Northern blot and quantifiable dot-blot analyses were performed at the end of the 48-h period using an anti-tau oligonucleotide probe (266-305 of the rat tau cDNA). Tau mRNA expression increased in cultures treated with either MAM alone (140% of controls) or glutamate alone (202% of controls). Cultures treated with MAM prior to glutamate showed the greatest increase (266% of controls). DNA damage (O^6-methylguanine) was detected by immuno-slot-blot analysis using anti-mouse O^6-alkylguanine monoclonal antibody (EM-21, kindly provided by Prof. M. Rajewsky, University of Essen, Germany) in a group of cultures treated for 12 h with MAM (100 μM) alone, followed by 24 h of control culture medium. These results show that MAM (1) produces persistent (36 h) modification of neuronal tau mRNA expression and (2) amplifies the elevated tau mRNA expression produced by glutamate. The role of MAM-induced DNA damage in altering tau mRNA expression is under study. [Supported by Ministaire de l'Enseignement superière et de la Recherche, and USPHS grant NS 19611]

D3-203 LINKAGE OF FAMILIAL SPASTIC PARAPLEGIA TO CHROMOSOME 15Q: PHENOTYPE/GENOTYPE CORRELATIONS. John K. Fink, Sandra M. Jones, C-T Brocade Wu, Greg B. Sharp, Bernadette Lange, Brith Otterud, Mark Leppert, Department of Neurology, The University of Michigan, Ann Arbor, MI 48109-0642
FSP refers to a group of disorders with the primary feature of progressive, severe, lower extremity spasticity. Recently, we performed genetic linkage analysis in a kindred with autosomal dominant, uncomplicated FSP. Affected subjects developed progressive gait disturbance between ages 12 and 35 and exhibited lower extremity hyperreflexia, spasticity, and weakness, extensor plantar response, diminished vibratory sense, and pes cavus. There was no clinical evidence of genetic anticipation. In our kindred, we observed close linkage between the disorder and microsatellite polymorphisms on chromosome 15q (eg. D15S128, LOD=9.70, θ =0.05). Multipoint linkage analysis reached a maximum LOD score (10.16) between D15S128 and D15S156. This region includes two candidate genes, α5 and β3 subunits of $GABA_A$ receptor. Currently, the FSP locus spans a 7.3 cM interval of proximal chromosome 15q and includes regions deleted in Prader-Willi (PWS) and Angleman (AS) syndromes. The fact that chromosome deletions responsible for PWS or AS do not produce progressive spastic paraplegia suggests that the FSP gene may act in a dominant negative manner. Recently, we obtained a series of overlapping YAC clones that span the FSP locus on chromosome 15q. This resource will expedite the discovery and analysis of FSP candidate genes.

The most important difference between our patients (linked to chromosome 15q) and those reported by Hazan *et al* (1993) (linked to chromosome 14q) is the severity of the illness. The disorder in Hazan *et al*'s kindred may be more mild in so far as only one patient (age 69-years) required a wheelchair. At least nine of our 31 affected subjects required a wheelchair (beginning for some as early as their late 40's). Despite this difference, our patients are quite similar to those reported by Hazan *et al.* This observation raises the possibility that the different abnormal gene products responsible for these disorders participate in a common biochemical cascade that results in a similar pattern of central nervous system degeneration.

D3-204 ACTIVATION OF PROENKEPHALIN GENE PROMOTER IN LYMPHOCYTES FROM TROPICAL SPASTIC PARAPARESIS PATIENTS, Jay B. Joshi*§, Harish P. Dave**†, Clarance J. Gibbs# Jr., and Robin Mukhopadhyaya+, *Molecular Biology and **Molecular Hematology Laboratories, VA Medical Center, Washington DC 20422; §Department of Microbiology and Immunology and †Division of Hematology, George Washington University, Washington DC 20037; #Laboratory of Central Nervous System Studies, NINDS, NIH, Bethesda MD 20892 and +Cancer Research Institute, Bombay India.
HTLV-I, an etiologic agent for adult T-cell leukemia/lymphoma (ATLL) causes tropical spastic paraparesis (TSP) or HTLV-I associated myelopathy (HAM), a chronic degenerative neurological disorder. The mechanism(s) of HTLV-I pathogenesis remains obscure and Tax1-a HTLV-I transacting protein- is often implicated as a crucial viral product influencing this process. We earlier reported the transactivation of the proenkephalin gene (ENK) promoter by the Tax1 protein in glial cells (Joshi & Dave, *Proc. Natl. Acad. Sci. USA.* 89: 1006-1010; 1992) . Proenkephalin is the precursor of opioids enkephalins that function as neurotransmitters. The proenkephalin gene is expressed in variety of cell types including lymphocytes and enkephalins have been implicated as neuroimmunomodulators. We investigated the expression of proenkephalin/CAT chimeric gene in lymphocyte cell-lines derived from TSP and ATL patients using transient transfection assays. TSP/HAM lymphocyte cell-lines were transfected with 10 μg pREJCAT(-2700/+53bp) DNA using DEAE-Dextran and 48 hours later CAT activity assayed. In HTLV-I infected lymphocytes from TSP and ATL patients, the ENK promoter driven CAT expression increased up to 16-fold over normal lymphocytes. To ascertain that the effect was Tax1-mediated (Tax1 stimulates the transcription of the HTLV-I genome by transactivation of the 5' LTR promoter), we also transfected these cells with HTLV-I LTR-CAT DNA. HTLV-I LTR driven CAT expression was stimulated up to 38-fold in TSP/ATL lymphocytes. This transactivation correlated with the titer of HTLV-I antigen expression in the infected cells. It is often proposed that the enkephalins may act directly at the inflammation site or indirectly as signal mediator between the nervous and immune systems. Therefore, it is conceivable that the Tax1-mediated transregulation of proenkephalin gene may serve as a molecular basis for bi-directional communication between the immune and neuroendocrine systems in HTLV-1 related disorders.

D3-205 CAG EXPANSIONS IN A NOVEL GENE FROM MACHADO-JOSEPH DISEASE AT HUMAN CHROMOSOME 14q32.1

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We have identified a novel gene containing CAG repeats and mapped it to chromosome 14q32.1, the genetic locus for Machado-Joseph disease (MJD). In normal individuals the gene contains between 13 to 36 CAG repeats, whereas most of the clinically diagnosed patients and all of the affected members of a family with clinical and pathological diagnosis of MJD show expansion of the repeat-number (from 68-79). This establishes MJD as the 8th inherited disease known to be associated with expansions of a trinucleotide repeat. The CAG repeat is in the coding region of a cDNA and is predicted to give rise to a long glutamine tract, and those patients with longer repeats have an early onset of disease. Although no homologous proteins were found, we have identified at least three related genes and have mapped them to different human chromosomes. These related genes could, if they also contain the CAG repeats, potentially cause similar disease which might be difficult to distinguish clinically from MJD. We are currently undertaking detailed structural analyses of these related genes and searching for novel CAG expansions.

D3-206 THE RELATIONSHIP BETWEEN TRINUCLEOTIDE REPEAT(CAG) LENGTH AND CLINICAL FEATURE IN MACHADO-JOSEPH DISEASE

Hideshi Kawakami, Hirofumi Maruyama, Zenjiro Matsuyama, Yoshiya Kawaguchi*, Akira Kakizuka*, Shigenobu Nakamura, and Co-study group**. Third Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima, 734, Japan and *Department of Pharmacology, Kyoto University Faculty of Medicine, Kyoto, 606-01, Japan

The initial observation of an expanded CAG trinucleotide repeat in Macado-Joseph disease has now been confirmed and extended in 78 MJD individuals from 40 independent Macado-Joseph disease family . Normal chromosomes displayed 14-34 repeats in MJD1a gene. 14 repeats is the most popular (35%) and the shortest in normal population. MJD1 chromosome contained 60-84 repeat units. The MJD1 repeat length was inversely correlated(n=78, r=0.87) with the age of onset .It accounts for approximately 76% of the variation in the age of onset. Homozygote showed the earlier onset expected from the regression curve of age of onset by length of CAG repeat. Parent-child pairs examined show the CAG trinucleotide expansions.

Co-study group**; Tetsuo Sakai (National Chikugo Hospital), Manabu Doyu, Gen Sobue (Aichi Medical University), Makiko Seto, Mitsuhiro Tsujihata (Nagasaki Kita Hospital), Takekazu Ohi (Miyazaki Medical College), Kenji Nishio (National Musashi Hospital), Ryosuke Takahashi (Tokyo Metropolitan Institute of Neuroscience), Michiyuki Hayashi, Ichizou Nishino, Toshiyuki Ootake (Tokyo Metropolitan Neurological Hospital), Tetsurou Oda, (National Shimofusa Sanatorium), Kimitaka Nishimura, Takahiko Saida (National Utano Hospital), Shushumu Chiba (Sapporo Medical College), Masayuki Baba (Hirosaki University)

D3-207 AN ANIMAL MODEL FOR STUDYING THE ROLE OF CYSTATIN C IN THE DEVELOPMENT OF CEREBRAL HEMORRHAGE, Hyung-Suk Kim, Department of Pathology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

Cystatin C is a cysteine protease inhibitor which protects cells from inappropriate endogenous or external proteolysis by cysteine proteases, such as the cathepsins. A mutation in the gene coding for cystatin C is known to be important in the etiology of hereditary cerebral hemorrhage with amyloidosis of the Icelandic type (HCHWA-I). Cystatin C is also co-localized with beta-amyloid protein on cerebral vessel walls of some patients with cerebral amyloid angiopathy.
To study the role of cystatin C in the central nervous system, I created inbred mice carrying precise mutation in the cystatin C gene. I have inactivated the mouse cystatin C gene in embryonic stem (ES) cells by homologous recombination. A 14 kb genomic fragment containing whole gene including exons 1, 2 and 3 was cloned from DNA of mouse strain 129 which was the source of the ES cells. Into the exon 3, a neomycin resistance (neo) gene was inserted after deleting 500 bp fragment, which contained stop codon and poly (A) additional site. At the 3' end of the fragment a thymidime kinase (tk) gene was added for negative selection. Using this construct, ES cells were transfected by electroporation. One hundred sixty five colonies in four separate experiments were screened by PCR analysis after selection G418 and ganciclovir. Twenty two PCR positive clones were obtained. Targeting was confirmed by genomic Southern blots showing that the targeting frequency was 1/8. One targeted ES cell has been generated several male chimeras after injecting into host blastocysts and reimplanting into foster mice. In order to obtain germ line transmission of the inactivated gene, chimeras are breeding. The histopathological study will be discussed with the cystatin C gene deficient mice: heterozygote (+/-) and homozygote (-/-).

D3-208 ISOLATION, CHARACTERIZATION, AND MAPPING OF MOUSE EXCITATORY AMINO ACID TRANSPORTERS (EAATS) M. A. Kirschner‡*,1, N.G. Copeland, D.J. Gilbert†, N. A. Jenkins†, & S. G. Amara‡, ‡The Vollum Institute for Advanced Biomedical Research, and *Department of Neurology, Oregon Health Sciences University, Portland, Oregon 97201; † ABL Basic Research Program, NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702.

Neurotransmitter reuptake by high-affinity transport molecules may modulate synaptic signaling by reducing extracellular neurotransmitter concentrations. Toxic effects ascribed to excitatory amino acids may also be prevented or limited by transporter activity. We have isolated mouse cDNAs and genomic clones for four sodium-dependent, high-affinity amino acid transporters(EAATs 1-4). There appears to be a high degree of interspecific nucleic acid and amino acid sequence conservation. Functional assays give comparable results when human and mouse transporters are compared. Tissue-specific expression is also conserved between species. Three of the four genes are relatively specific for their expression within the nervous system. There is regional variation of transcript abundance in the brain.

We have mapped the mouse EAAT genes using interspecific backcross analysis. *EAAT1* maps to mouse chromosome 15. While there do not appear to be known mouse mutants linked to *EAAT1* the human EAAT1 gene has been mapped to a syntenic region of human chromosome 5 (5p13) the deletion of which has been associated with human microcephaly and mental retardation. *EAAT2* maps to mouse chromosome 2 in the vicinity of a quantitative trait locus for the epilepsy strain EL (*EL-2*). *EAAT3* maps to mouse chromosome 19. EAAT genes are of particular interest because of the association of diminished glutamate transport with both human and mouse motor neuron diseases. Studies of glutamate transport in the mouse model will contribute to our understanding of the role of the high-affinity glutamate transporter in the biology of the motor neuron and the pathogenesis of motor neuron disease.

D3-209 REACTIVE OXYGEN SPECIES PRODUCED BY THE CYCAD TOXIN METHYLAZOXYMETHANOL, A CANDIDATE ETIOLOGICAL FACTOR FOR WESTERN PACIFIC ALS/P-D. †G.E. Kisby, ΔD. Eizirik, †C. Sweatt, †P.S. Spencer. †Center for Research on Occupational and Environmental Toxicology; Oregon Health Sciences Univ., Portland, OR 97201. ΔDepartment of Medical Cell Biology, Uppsala Univ., Uppsala, Sweden.

Cycasin, through its metabolite methylazoxymethanol (MAM), is a potential trigger for western Pacific amyotrophic lateral sclerosis (ALS) and Parkinsonism-dementia (P-D) complex [Kisby *et al.* Neurodegeneration 1:73-82, 1992], a prototypical neurodegenerative disorder associated with diabetes mellitus (ALS) or carbohydrate intolerance (P-D) [Koerner Diabetes 25:1055, 1976]. MAM is a direct-acting DNA toxin that perturbs gene expression *in vitro* in association with persistent alterations in post-mitotic neuronal DNA. DNA damage may result from the direct alkylation of nucleotides (*see* Esclaire *et al.*, this meeting) or indirectly *via* the formation of free radicals. Cycasin/MAM is proposed to act as a "slow toxin" [Spencer *et al.*, Neurology 41:62-66, 1992] by initiating neuronal (DNA) damage that is subsequently promoted by an endogenous agent such as glutamate. The present study explores the molecular mechanisms underlying MAM-induced DNA damage, in particular the possibility that the cycad toxin forms reactive oxygen species. Related azoxy compounds (procarbazine) are known to produce free radicals *in vitro* and *in vivo*. To test for the generation of specific free radicals, MAM acetate (0.1 mM, 1 mM) was incubated at 37°C for 24 h with either SY5Y human neuroblastoma or pancreatic β-islet culture media and nitrite levels (a measure of the nitric oxide free radical NO•) determined colorimetrically using the Greiss reagent. Nitrite levels were 2-10 fold higher in 1 mM *vs* 0.1 mM MAM-treated media. MAM depressed glucose oxidation in mouse β-islet cells, and this depression was attenuated by free-radical scavengers (dimethylsulfoxide > hemin). Additionally, treatment of SY5Y human neuroblastoma cell cultures for 1h with 1 mM MAM resulted in a significant increase (2.5 X control) in cellular hydrogen peroxide levels, as determined by fluorescence detection of dichlorofluorescein in cultures treated with dichlorofluorescin. Levels of hydrogen peroxide were similar to those found in SY5Y cells comparably treated *in vitro* with 5 μM hydrogen peroxide. These data suggest MAM (1) increases free radicals in human neurons and mouse β-islet cells and (2) potentially induces oxidative stress and DNA damage. [Supported by NS19611 and Medical Research Foundation of Oregon]

D3-210 CLONING OF A STRONG CANDIDATE GENE FOR SPINAL MUSCULAR ATROPHY (SMA) WITH HOMOLOGY TO BACULOVIRUS PROTEINS THAT INHIBIT APOPTOSIS, Robert G. Korneluk[1], Mani S. Mahadevan[1], Natalie Roy[1], Michael McLean[1], Zahra Yaraghi[1], Reza Farahani[1], Katsuyuki Tamai[1], Thomas O. Crawford[2], Pieter de Jong[3], Linda Surh[1], Joh-E Ikeda[1] and Alex E. MacKenzie[1]. [1]Molecular Genetics, Children's Hospital of Eastern Ontario, Ottawa, Canada K1H 8L1, [2]Human Genetics Department, Roswell Park Cancer Institute, Buffalo, New York 1423 USA, [3]Department of Neurology, John Hopkins University, Baltimore, Maryland, USA

The childhood spinal muscular atrophies (SMA) are common autosomal recessive disorders characterized by a depletion of spinal cord motor neurons that result in denervation atrophy of skeletal muscles. Childhood SMA is the most common monogenic cause of death in infancy. Apoptosis of motor neurons is a well-documented phenomenon in normal embryogenesis; one model of SMA pathogenesis invokes an inappropriate persistence or reactivation of apoptosis. In support of this hypothesis, we have identified a novel gene that maps to the SMA containing region of chromosome 5q13.1, designated NAIP for neuronal apoptosis inhibitory protein. NAIP contains domains with homology to baculoviral apoptosis inhibitor proteins. The SMA chromosomal region contains, in addition to the intact NAIP gene, a variable number of differentially deleted copies. RT-PCR analysis and cDNA library screening suggest that the intact NAIP gene and its deleted copies are transcribed complicating the analysis of this gene for mutations potentially causative of SMA. Nevertheless, SMA chromosomes appear to have more extensive loss or deletions of NAIP gene copies: for example, we find that the first two exons of the intact NAIP gene are absent in approximately 67% of Type I SMA chromosomes compared to 2% of non-SMA chromosomes. Analysis of other exons shows similar depletion in SMA individuals. We suggest that the NAIP gene(s) is a strong candidate for SMA and that mutations in this locus may lead to a failure of a normally occurring inhibition of motor neurons apoptosis.

D3-211 EXPRESSION OF A MUTANT NEUROFILAMENT SUBUNIT LEADS TO EXTENSIVE MOTOR NEURON DEATH, Michael K. Lee*, Joseph Marszalek*, and Don W. Cleveland*,+, Departments of Biological Chemistry* and Neuroscience+, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Abnormal accumulation of neurofilaments have been implicated in the etiology of human motor neuron diseases, and the overexpression of neurofilament subunits in the transgenic mouse models can lead to motor neuron dysfunctions. However, whether the abnormalities in neurofilaments ultimately leads to neuronal loss is not clearly established. We now show using transgenic mice technology that abnormalities in neurofilaments can lead to extensive loss of motor neurons from the spinal cord. In transgenic mice where the assembly disrupting mutant NF-L protein accumulated to approximately 50 % of endogenous NF-L in the sciatic nerve, the mice exhibits severe weakness in both fore- and hind-limbs. Both light and ultrastructural level examinations reveal an extensive motor axon degeneration in the peripheral nerves and wide-spread signs of large motor neuron death in the spinal cord. In the few surviving motor neurons, parakaryal and axonal accumulations of NFs are evident. Morphometric analysis of vnetral axons reveals the selective loss of largest motor neurons. The neuronal pathology is accompanied by severe denervation atrophy of the skeletal muscles. Thus, expression of the mutant NF subunits in transgenic mice produces virtually the entire range of clinical symptoms and the cellular pathology seen in most human motor neuron disease, including amyotrophic lateral sclerosis.

D3-212 THE LETHAL CONGENITAL CONTRACTURE SYNDROME- A MOTONEURON DISEASE OF THE FETUS, Päivi Mäkelä-Bengs*, Katri Vuopala**, Anu Suomalainen*, Leena Peltonen *, * Department of Human Molecular Genetics, National Public Health Institute, FIN-00330 helsinki, ** Department of Pathology, University of Oulu, FIN-90220 Oulu.

The aim of this study is to assign the gene locus and clarify the molecular pathogenesis of lethal congenital contracture syndrome (LCCS), an autosomal recessively inherited syndrome (McKusick 253310) leading to perinatal death. Early-onset degeneration of the anterior horn motor neurons of the spinal cord is a typical neuropathological finding in LCCS fetuses. Identification of gene locus and LCCS mutation will provide us with new insight into development of motoneurons.

The neuropathological findings in the LCCS closely resemble those in spinal muscular atrophy (SMA). However, linkage analysis of 33 samples from seven LCCS families, including ten affected fetuses, excluded the SMA 5q region as the chromosomal locus for the LCCS.

Exaggeration of naturally occurring motoneuron death (physiological apoptosis) is proposed to contribute to the anterior horn damage. In LCCS, this mechanism could play an important role, because degeneration of the anterior horn motor neurons in the LCCS fetuses overlaps the physiological apoptosis phase in the motor neurons during fetal development. Some of the genes involved in apoptosis have been localized on specific chromosomes as have been the genes for neuronal growth factors and their receptors, which are expressed during the development of the nervous system of fetuses. Linkage analyses between the LCCS and these loci are in progress as well as the random genome search using highly informative polymorphic markers to assign the chromosomal locus for LCCS.

D3-213 **HAIRPIN STABILITY DETERMINES THE THRESHOLD AND POTENTIAL FOR NUCLEOTIDE EXPANSION ASSOCIATED WITH HUMAN NEURODEGENERATIVE DISEASE,**

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The expansion of di- and tri-nucleotide repeating units has been identified as an important new class of human mutations that are the genetic basis for several neurodegenerative disorders. The mechanism by which these expansions arise is unknown. Based on stability and frequency analysis, our data indicate that large expansions associated with human disease are dependent on the ability of that region to form a stable hairpin structure. Hairpin stability has two components, base sequence and length. Only hairpins of sufficient length and the correct sequence will have the potential to form stable enough structures for amplification. Within the context of the disease genes, repeat regions form hairpins of common energy although the number and sequence of the repeat may vary. The threshold hairpin energy required for expansions roughly -45 kcal/hairpin. Below this energy, the hairpins are unstable and it is unlikely that expansion can occur. However, the repeat copy number that determines the threshold can be smaller if flanking sequence increases hairpin stability. NMR data reveals that hairpins form with mismatched base pairs form within CAG/CTG repeat oligonucleotides. The mispaired bases are highly stacked within the stem of the duplex. Our model predicts the expanded sequences should be CAG, CGG, CA repeats- all those that are currently found associated with human disease. Hairpin stability predicts the sequence selectivity of expansion, predicts the length dependence of expansion, provides a structural basis for the constancy of the CCG region in Huntington's disease and explains the stabilizing effects of AGG interruptions in FMR-1 alleles.

D3-214 ABERRANT CYTOSKELETAL PHOSPHORYLATION AS A PATHOGENIC MECHANISM IN NEURODEGENERATIVE DISEASES -MECHANISMS OF CYTOSKELETAL PHOSPHORYLATION STUDIED IN CULTURED CELLS AND TRANSGENIC ANIMALS.

Christopher C.J. Miller, Janet Brownlees, Barry Gibb, Sonia Guidato, Nick Irving, Simon Lovestone, Uta Wagner, Brian Anderton and Jean-Marc Gallo. Departments of Neuroscience, Neurology and Old Age Psychiatry, The Institute of Psychiatry. Denmark Hill, London SE5 8AF UK.

Changes in phosphorylation of the neuronal cytoskeleton are seen in a number of neurodegenerative diseases. In particular, aberrant phosphorylation of the microtubule-associated protein (MAP) tau is observed in Alzheimer's disease and perikaryal accumulations of phosphorylated neurofilaments are observed in Motor Neurone disease. We have investigated the mechanisms and role(s) of MAP and neurofilament phosphorylation using transfected cells and transgenic mouse technology. Many of the phosphorylation sites in these proteins are in a proline environment and so we have studied the effect of elevating activity of the proline directed kinases GSK-3α and GSK-3β, p42 and p44 MAP kinase and cdk-5 on MAP and neurofilament phosphorylation. By such manipulations and by mutating known phosphorylation sites in both MAPs and neurofilaments, we have identified cellular kinases responsible for phosphorylating some MAPs and neurofilaments. Our results also provide insight into the role(s) of cytosketetal phosphorylation in nerve cell biology.

D3-215 LOWER MOTOR NEURONE DISORDER FOLLOWING USE OF A MOSQUITO REPELLANT

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From 1975 to 1981 a product containing 16.67% dimethylphthalate (DMP) and 8.33% 2-ethyl, 3-hexanediol (EHD) in alcohol 36 and 60% was marketed. The product was used on the golf course and applied as instructed to the right hand, then distributed to the head and neck area. Muscous membranes were avoided. Use was sometimes associated with lip numbness and a warm feeling in recipient skin. The Merck Index confirms that (DMP) is a skin irritant and has C.N.S. toxicity. (EHD) is also a neurotoxin capable of percutaneous absorption. Together they exert a synergistic toxic effect. The alcohol in the repellant promotes skin absorption since it delipidizes skin.

Four years after commencing the use of this product a 56 year old presented with weakness in the right hand. Examination showed atrophy in the muscles of the right hand, forearm and arm. Myelograms were normal. A neurologist and a neuro-surgeon felt that amyotrophic lateral sclerosis was responsible. The subject avoids products like tobacco, alcohol and drugs with known neurotoxic effects. Positive mental imaging and autogenic programming are utilized to maximize healing propensities. Alfalfa sprouts, a rich source of Thyroid Releasing Hormone (TRH) are consumed daily. High doses of Vitamin C (25 gms) administered intravenously in 500 ml of 5% dextrose and water, given at weekly intervals effectively flush out free radicals from the brain and body, diminishing degeneration and enhancing healing. Over the course of ten years there has been improvement in muscle strength in the right hand and forearm. The turning of a key and golf proficiency are personalized performance parameters to document improving status. Consumers should be alerted to the action of products offered for sale without prior testing in primates, which may repel insects but are lethal to human cells. Application of insecticides to garments or hair bearing parts discourages systemic absorption.

D3-216 TIRILAZAD MESYLATE (U-74006F) PROTECTS MOTOR NEURONS IN THE FACIAL NUCLEUS FOLLOWING TRANSECTION OF THE FACIAL NERVE IN NEONATAL RATS,

S.L. Smith, J.A. Oostveen & E.D. Hall, CNS Disease Research, The Upjohn Company, Kalamazoo, MI

Recent reports suggest that the motor nerve degeneration characteristic of amyotrophic lateral sclerosis may result from a genetically-induced increase in sensitivity to the damaging effects of oxygen radicals. A likely mechanism by which free radicals damage motor neurons is through membrane degradation via lipid peroxidation. The purpose of the present study was to test the ability of the antioxidant, lipid peroxidation inhibitor, tirilazad mesylate (U-74006F) to protect cholinergic cell bodies (motor neurons) in the facial nucleus following facial nerve axotomy in 14-day-old rat pups. On Day 1, the right facial nerve of each rat was transected at its point of exit from the stylomastoid foramen. Pups were pretreated orally with either 10 or 30 mg/kg U-74006F or cyclodextrin vehicle, 10 minutes before axotomy and post-treated once a day from Day 2-7, and then once every other day from Day 8-21. Rats were sacrificed three weeks post-transection and the surviving motor neurons, identified through choline aceytltransferase immunocytochemistry, were counted. In vehicle-treated rats, 55.1% of the motor neurons in the ipsilateral facial nucleus survived 21 days following facial nerve axotomy in comparison to the nonaxotomized contralateral side ($p<.0001$). Pre- and post-treatment with 10 and 30 mg/kg U-74006F significantly enhanced motor neuron survival, with survival rates of 71.0 and 72.3%, respectively ($ps<.0001$ vs axotomized vehicle controls). The fact that the antioxidant, lipid peroxidation inhibitor, U-74006F, protected cholinergic cell bodies in the facial nucleus from post-axotomy degeneration lends support to the notion that free radical damage may be a fundamental mechanism involved in motor neuron degeneration.

D3-217 TRANSGENIC AND CELL CULTURE MODELS OF FAMILIAL AMYOTROPHIC LATERAL SCLEROSIS LINKED TO MUTATIONS IN SUPEROXIDE DISMUTASE 1, Philip C. Wong[1], Carlos Pardo[1], David R. Borchelt[1], Mike K. Lee[4], Zuo-Shang Xu[4], Michael Guarnieri[1], Hilda H. Slunt[1], Nancy A. Jenkins[5], Neil G. Copeland[5], Sangram S. Sisodia[1], Donald L. Price[1-3] and Don W. Cleveland[3,4], Departments of [1]Pathology, [2]Neurology, [3]Neuroscience and [4]Biological Chemistry, The Johns Hopkins University School of Medicine, Baltimore, MD 21205; [5]Mammalian Genetics Laboratory, Frederick, MD 21703

Mutations in the Cu/Zn superoxide dismutase (SOD1) have recently been identified in a subset of individuals with autosomal dominant familial amyotrophic lateral sclerosis (FALS). We show that four lines of transgenic mice expressing one of these missense mutations with a Gly^{37}-> Arg substitution, at a protein level ranging from 4 to 8 times the endogenous mouse SOD1 in the spinal cord develop a clinical-pathological phenotype of motor neuron disease. At 4 to 6 months, animals develop hindlimb weakness and muscle atrophy; eventually, the mice become paralyzed and die from 5 to 12 weeks after onset of disease. Examinations of mice at 10 to 12 weeks of age show vacuolar degeneration, primarily of mitochondria, in the soma, dendrites, and axons of motor neurons, accompanied by neurofibrillary axonal pathology. At 4 to 5 months of age, there is evidence of reductions in the number of motor neurons, degeneration of axons in the ventral roots, and denervation-induced muscle atrophy. Mice expressing the wild-type human SOD1 up to 10 times the endogenous SOD1 do not exhibit disease and the G37R mutant enzyme does not show reduced levels of SOD1 activity. These results indicate that the G37R mutation exhibits a gain of an adverse property, which leads to motor neuron disease. Using transient and stable gene expression systems, we show that the G37R mutant polypeptide retains significant activity. Mutant SOD1 subunits do not affect the activity or the longevity of wild-type subunits indicating that the mutant SOD1 is not a direct antagonist of wild-type enzyme. We conclude that mutant SOD1 acquire a neurotoxic property, the dominant effect of which is central to the pathogenesis of this type of FALS.

Genetics of Alzheimer's Disease; Ad and Prion Models

D3-300 STRAIN SPECIFIC CELL-FREE FORMATION OF THE PRION PROTEIN, Richard A. Bessen, David A. Kocisko, Gregory J. Raymond, Richard F. Marsh, Peter T. Lansbury, and Byron Caughey, Laboratory of Persistent Viral Diseases, Rocky Mountain Labs, National Institute of Allergies and Infectious Diseases, Hamilton, MT. 59840, Dept. of Chemistry, Massachusetts Institute of Technology, Cambridge, MA. 02139, Dept. of Animal Health and Biomedical Sciences, University of Wisconsin, Madison, WI. 53706

Transmissible mink encephalopathy (TME) causes a progressive neurodegeneration in ranch-raised mink similar to scrapie in sheep. Brain PrP^{TME} from the HY and DY strains of TME have distinct conformations based on differences in sedimentation properties, the rate of digestion with proteinase K (PK), and the PK N-terminal cleavage sites. To investigate the strain specificity of PrP^{TME}-PrP^{C} interactions we used a cell-free system to measure the conversion of the PK sensitive PrP^{C} to PK resistant PrP. Incubation of hamster ^{35}S-methionine labeled PrP^{C} with HY or DY PrP^{TME} followed by PK digestion resulted in PK resistant ^{35}S-PrP conversion products. The properties of the PrP conversion products were determined by the strain of PrP^{TME}; the strain differences between the PK resistant PrPs formed in vitro were similar to those found in brain PrP^{TME}. Each strain of PrP^{TME} appeared to act as a distinct template for *de novo* PrP^{TME} formation, suggesting that PrP^{TME} conformation is the molecular basis of strain variation.

D3-301 DRUGS THAT INCREASE SCRAPIE INCUBATION PERIOD DISRUPT NUCLEOPROTEIN COMPLEXES IN VITRO, Lynne Borchardt-Sendek, Jean Kaczkowski, Judd Aiken and Debbie McKenzie Department of Animal Health and Biomedical Science, University of Wisconsin, Madison, WI 53706.

Scrapie, a transmissible spongiform encephalopathy (TSE), is a neurodegenerative disease characterized by long incubation periods, insidious onset of symptoms and inevitable fatality. Although little is known of the molecular pathogenesis, a host-encoded protein, the prion protein (PrP), is important for susceptibility and accumulates during the disease as an abnormal isoform.

Preparations enriched for PrP can be treated extensively with nucleases and zinc without loss of infectivity - yet they contain significant amounts of nucleic acids, suggesting that the nucleic acids are protected. Through the use of electrophoretic mobility shift assays (EMSA), we have identified nucleic acid binding activities in PrP-enriched preparations. Comparison of these infectious preparations with similar preparations from uninfected animals reveals an apparent difference in size of the nucleoprotein complex between the two.

Several drugs known to prolong scrapie incubations period *in vivo* (Amphotericin B, carrageenan, Congo red, dextran sulfate, HPA-23, pentosan polysulfate) were tested for effects *in vitro*. Those drugs with *in vivo* antiscrapie activity prevented formation of nucleoprotein complexes in the EMSA, while those ineffective *in vivo* (dextran) were also ineffective *in vitro*. Mixing of drugs effective in the EMSA with scrapie agent inoculum resulted in prolonged incubation periods compared to control animals. Testing of related compounds in our assay may uncover new treatments for the TSEs and help determine the mechanisms of action of the drugs.

D3-302 PROCESSING OF THE ALZHEIMER'S AMYLOID PRECURSOR PROTEIN IN TRANSGENIC MICE, D. Borchelt[1], G. Thinakaran[1], R. Johannsdottir[2], D. Price[1,3,4], S. Sisodia[1], and Karen Hsiao[2], [1,3,4]Departments of [1]Pathology, [3]Neuroscience, and [4]Neurology, The Johns Hopkins School of Medicine, Baltimore MD 21205; 2Department of Neurology, University of Minnesota School of Medicine, Minneapolis, MN 55455.

Early-onset familial Alzheimer's disease (FAD) has been linked to mutations in the Aß amyloid precursor protein (APP). We have generated a number of transgenic FVB mice that express wild-type or mutant human APP (HuAPP-695, V642I, V646A, M647V), and examined the processing of these proteins in vivo. Mouse neuroblastoma N2a cells transfected with mutant APP secrete 3-fold more soluble Aß into culture medium as compared to wild-type APP . Mice that express the mutant human APP at levels equivalent to, or higher than, endogenous APP die prematurely and frequently develop gliosis in the cortex and hippocampus. Wild-type APP transgene product appears to be less toxic as abnormalities are only observed when the level of wild-type APP transgene product exceeds that of mouse APP by at least 2-fold. Using a variety of specific APP antibodies in immunoprecipitation and immunoblotting approaches, we have begun to compare the processing of APP in transgenic mouse brain to the events occurring in human brain. In both normal and AD human brain, we find that a significant fraction of accumulated APP appears to have been cleaved by α- and/or β- secretase. In contrast, in transgenic FVB mouse brain, we do not detect α/β secretase-cleaved APP. Moreover, we are unable to detect soluble Aß in extracts of brain from transgenic FVB mice that express either wild-type or mutant human APP, even in cases in which the level of human APP per μg of total protein in transgenic mouse brain is 5-10 fold higher than human brain. Whether FVB mice lack secretase activities, or whether cleaved APP molecules are rapidly cleared from the brains of these mice remains to be determined.

D3-303 OVERPRODUCTION OF A FRAGMENT OF AMYLOID PRECURSOR PROTEIN RESULTS IN THE DETECTION OF THE C-TERMINAL COMPLEMENT OF β-AMYLOID PEPTIDE, Abraham M. Brown, Donna M. Tummolo, Michael A. Spruyt, June Sonnenberg-Reines and J. Steven Jacobsen, Department of Central Nervous System Biological Research, Medical Research Division, Lederle Laboratories, Pearl River, New York 10965

It has been proposed that proteolytic fragments of amyloid precursor protein (APP) containing the beta amyloid protein (βAP) sequence are biochemical intermediates in the formation of βAP. Previously, we reported that overexpression of a membrane-spanning, βAP-containing fragment (C_t-102) in cultured cells resulted in increased release of soluble βAP. We now demonstrate that overexpression of C_t-102 also results in the appearance of a smaller carboxy-terminal (C-terminal) containing fragment, with apparent molecular weight of ~7 kDa, which is complementary to the released βAP. Radiosequence analysis indicates that the amino-terminus of this fragment begins with $βAP_{40}$. Treatment of cells with brefeldin A or carbonyl cyanide m-chlorophenylhydrozone results in up to a 10-fold increase in the ratio of ~7 kDa fragment to C-102 in a titratible manner. Cellular fractionation experiments indicate that both C_t-102 and the ~7 kDa fragment are membrane bound. Taken together, these findings suggest that γ-secretase activity is located in the endoplasmic recticulum (ER) and is able to cleave a peptide sequence that is ordinarily buried within the lipid membrane. Pulse-chase experiments indicate that the C_t-102 is turned over more rapidly than full length APP, suggesting that there is a stabilizing determinant within the extracellular domain of APP that is absent in C_t-102.

D3-304 THE SECRETION OF ALZHEIMER'S BETA AMYLOID PRECURSOR PROTEIN IN MICROGLIA IS REGULATED BY cAMP. Richard T. Carroll, Jennifer L. Grimm and Brenda D. Shivers, Neurological and Neurodegenerative Diseases Group, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105

Emerging evidence suggests that Alzheimer's disease (AD) has a neuroinflammatory component. Microglia are brain-specific macrophages which have been implicated in the destruction of neurons in Alzheimer's disease, Down's Syndrome and HIV dementia. Activated microglia can kill neurons by producing reactive oxygen species, glutamate, cytokines, nitric oxide as well as unidentified toxins that act on the N-methyl-D-aspartate receptors. Recently, the neurotoxicity of reactive microglia were found to be downregulated by ß-adrenergic agonists which increased cAMP levels. Interestingly, the human immunodeficiency virus coat protein gp120 was found to inhibit ß-adrenergic regulation resulting in decreased cAMP levels. To examine some potential roles of cAMP in neurodegeneration, we determined its effects on amyloid precursor protein (APP) secretion and nitric oxide synthase (NOS) activity in a mouse-derived microglia cell line (BV-2). When these microglia were activated with lipopolysaccharide (LPS), a 5-fold increase in APP secretion was detected along with the induction of NOS activity. Upon treatment with forskolin, isobutyl methylxanthine (IBMX) or Br-cAMP which raise intracellular cAMP levels, we found that there was no effect on NOS induction in either control or lipopolysaccharide (LPS)-activated microglia. However, increases in cAMP induced a 5-10 fold increase in APP secretion in both control and activated microglia. If APP acts as a neurotrophic factor as was suggested recently, then an increase in APP secretion may help prevent neuronal death. When adenylate cyclase was inhibited with dideoxyadenosine (DDA), secreted APP levels were reduced approximately 5-fold in both somnolent and activated microglia. DDA had no effect on NOS activity under any conditions. Our data demonstrate that the secretion of APP in microglia can be regulated by altering cAMP levels and that the regulation of adenylate cyclase activity may play an important role in neurodegenerative diseases.

D3-305 INHIBITION OF GLUTAMATE TRANSPORTER BY 25-35 β-AMYLOID PROTEIN FRAGMENT IN HUMAN RETINOBLASTOMA CELL, Crosson, C.E. [1,2], Ganel, R.[2], and Ittah, A.[1], Departments of Ophthalmology[1] and Pharmacology[2], Texas Tech University Health Science Center, Lubbock, TX 79430

In glutaminergic neurons, the end of the synaptic transmission requires the removal of glutamate from the synaptic cleft via high-affinity transporters. Also, the maintenance of low extracellular glutamate protects neurons from excitotoxic damage. This study characterizes glutamate transport in human retinoblastoma cells and the effect of 25-35 β-amyloid protein fragment (βAPF) on glutamate transport activity. Cell cultures were maintained in RPMI 1640 media containing 10% fetal calf serum. Kinetic analysis of the unidirectional glutamate uptake demonstrated that these cells express a high-affinity glutamate transporter with a Km of 2.1± 0.29 μM and a Vmax of 3.2 pmoles/minute/10^6 cells. Glutamate uptake was dependent upon extracellular Na^+ and intracellular K^+. Glutamate uptake was blocked by the inhibitors DL-threo-β-hydroxyaspartate (10^{-7}M) and dihydrokainate (10^{-7}M), but not by α-methyl-DL-glutamate (10^{-4}M). The incubation of retinoblastoma cells with 10^{-6} M βAPF for 15 minutes reduced the glutamate uptake, at 2 μM, by 51% when compared to control ($P < 0.05$). The EC_{50} for this response was 2.5×10^{-9} M. These data demonstrate that the human retinoblastoma cell line express a high-affinity glutamate transporter similar to that characterized from mammalian cortex. The addition of βAPF produced a significant dose-related reduction in glutamate transport activity. These data provide evidence that β-amyloid protein and/or a metabolite may function as an endogenous modulator of glutamate uptake. In Alzheimer's disease, the memory impairment and neuronal degeneration associated with β-amyloid protein may be mediated in part by the inhibition of glutamate transport activity.

D3-306 ALTERED EXPRESSION OF TRANSFORMING GROWTH FACTOR-ß IN ALZHEIMER'S DISEASE, Kathleen C Flanders, Carol F Lippa, Daniel A Pollen and Michael B Sporn, Laboratory of Chemoprevention, National Cancer Insititute, Bethesda, MD 20892 and Departments of Neurology and Pathology, University of Massachusetts Medical Center, Worcester, MA 01655

Three isoforms of transforming growth factor-ß (TGF-ß) are widely expressed in mammalian tissues where they regulate cell growth and differentiation and play a role in tissue repair. In some instances TGF-ßs have been shown to have neuroprotective effects. To determine if there is altered expression of TGF-ßs in Alzheimer's disease (AD), we performed immunohistochemistry with antibodies specific for each isoform of TGF-ß (TGF-ßs 1, 2, and 3) on brain tissue from patients with nondominantly inherited AD (n=9), autosomal dominantly inherited AD with linkage to 14q24.3 (n=4), and cognitively normal controls (n=10). There was limited staining for TGF-ß1 in all patients. Cytoplasmic staining of neurons with anti-TGF-ß3 was of similar intensity and occurred in a similar distribution in all AD and control samples. In contrast there was widespread staining of TGF-ß2 in glia from AD patients as compared to controls. Large tangle-bearing neurons also reacted with anti-TGF-ß2. A sandwich ELISA showed that extracts of brain tissue from AD patients contained 4.5 times more TGF-ß2 than did those from control patients. These data suggest selective induction of TGF-ß2 in AD. Studies with primary cultures of cortical neurons are being conducted in attempts to determine the functional significance of increased TGF-ß2 expression.

D3-307 PATHWAYS OF AMYLOID Aß CATABOLISM IN CULTURED CELLS. Charles Glabe, Debra Burdick and Austin Yang. Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717.

The concentration of any macromolecule, such asthe amyloid Aß peptide, is determined by its relative rates of production and degradation. We have examined the catabolism of Aß and its precursor protein, APP, in a variety of cultured cells. Exogenous Aß adsorbs to the surface of cultured human skin fibroblasts, transfected 293 cells and differentiated PC12 cells and is internalized at rates that are proportional to the surface adsorption. Internalized Aß1-42 has a different fate than intracellular Aß1-40 and shorter analogs. Intracellular Aß1-40 has a half life of approximately 1 hr in PC12 cells, while greater than 80 % of the intracellular Aß1-42 persists for 84 hr. The intracellular Aß1-42 fraction colocalizes with lysosomal markers and is largely sedimentable after solubilization of the cells in non-ionic detergents. Since lysosomes are a site of APP catabolism, we examined the effect of Aß1-42 on the catabolism of APP and APP fragments. The amounts of C-terminal, amyloidogenic fragments in stably-transfected 293 expressing APP are dramatically enhanced by treatment of the cells with Aß1-42, but not Aß1-28, which does not accumulate in cells. The amyloidogenic fragments are concentrated in the same detergent-insoluble fraction of the cell as the intracellular Aß1-42. The continued presence of exogenous Aß1-42 in the medium or adsorbed to the cell surface is not required for the stimulatory effect. Pretreatment of cells with Aß1-42, followed by removal and replacement with medium lacking peptide also results in accumulation of the amylodogenic fragments. Pulse-chase measurements indicate that the half life of the amyloidogenic fragments is greatly increased in the presence of Aß1-42, suggesting that their accumulation is due to enhanced stability. Our results suggest that intracellular Aß1-42 aggregates may establish a new pathway for APP catabolism in cells which leads to the long-term stability of the amyloidogenic fragments. If the fragments are further processed to Aß, this pathway could promote the accumulation of insoluble amyloid. Supported by NIH NS31230 and AG00538

D3-308 **DETECTION AND CHARACTERISATION OF BETA-SECRETASE CLEAVED APP FROM CELL LINES.**

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Proteolytic processing of the amyloid precursor protein (APP) gives rise to the βA4 peptide, which is deposited in the form of amyloid in the brains of patients with Alzheimer's disease. This peptide is thought to be derived from APP by the sequential action of β- and γ-secretases. The β-secretase cleavage also releases a large, soluble N-terminal fragment of APP (sAPPβ) into the extracellular environment. While several groups have now reported the secretion of βA4 by various cells in culture, evidence for the accumulation of sAPPβ in culture media is much more limited. We have generated a monoclonal antibody to a peptide corresponding to the C-terminus of sAPPβ (amino acid sequence = ISEVKM). Reaction with this antibody was shown to be dependent on the presence of a free C-terminal methionine. The antibody was used to characterise secretion of sAPP β by undifferentiated and differentiated neuroblastomas, and by APP-transfected cell lines. One major band of approximately 110 kD was identified in cell-conditioned medium by Western blotting. This protein was immunoprecipitated from culture medium, and its immunochemical profile (including lack of immunoreactivity with anti-bA4 antibodies) was shown to be consistent with an identity of sAPPβ. The 110 kD band was increased in cells transfected with APP, and was found to react with antibodies raised to the Kunitz-inhibitor domain of APP. This suggests that the APP695 isoform is not the primary substrate for β-secretase cleavage in the cell model investigated.

D3-309 STRUCTURAL BASIS OF PRION DISEASES

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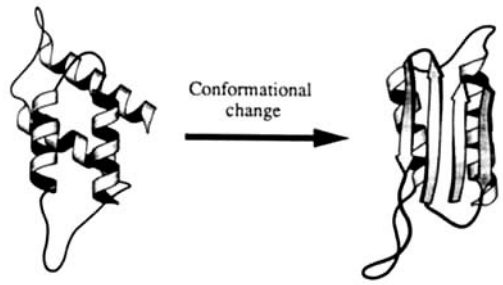
Prion diseases are a group of neurodegenerative disorders in humans and animals that seem to result from the conformational change of the normal cellular prion protein (PrP^C) into its abnormal infectious isoform (PrP^{Sc}). To characterize this conformational conversion process, a computational approach of combining molecular modeling techniques and spectroscopic and genetic data has been used to propose the three-dimensional structures of both PrP^C and PrP^{Sc}. In the absence of X-ray or NMR data, these predicted structures provide testable models for the three-dimensional structures of PrP^C and PrP^{Sc}. Furthermore, these structures reveal a plausible molecular mechanism for prion replication. As shown below, prion replication involves a process in which two α-helices of PrP^C are converted into a 4-strand β-sheet of PrP^{Sc}. Using the β-sheet as a template to direct this conformational change, infectious PrP^{Sc} could promote the conversion of noninfectious PrP^C to generate more PrP^{Sc} (prion multiplication). These results may provide a structural basis for prion diseases and the development of novel therapeutic strategies.



D3-310 IDENTIFICATION OF CELLULAR PROTEINS THAT ASSOCIATE WITH β-AMYLOID PEPTIDE. S. R. Hughes, P. Gonzalez-DeWhitt, S. Goyal, M. A. Fortes, N. Riedel and S. R. Sahasrabudhe. Department of Molecular Neurobiology, Hoechst-Roussel Pharmaceuticals Inc., PO Box 2500, Somerville, NJ 08876.

Alzheimer disease is characterized by the presence of neuritic plaques in the brain containing aggregates of Aβ peptide, a 39-42 amino acid fragment of the Amyloid Precursor Protein (APP). Since Aβ is found in the cerebrospinal fluid and plasma of normal individuals, it is hypothesized that there may be proteins that maintain Aβ in soluble conformation. Aβ may also have a physiological function via its interaction with other proteins. We use the interaction trap or two hybrid system in an attempt to clone cDNAs which encode proteins that interact with the Aβ peptide. The method uses the transcription of yeast reporter genes as a phenotype to detect protein-protein interactions (Gyuris *et al.*, Cell **75**: 791-803, 1993). The Aβ peptide is expressed as a Aβ-lexA fusion protein. Such a fusion protein can bind to LEU2 or lacZ reporters that have their upstream regulatory sequences replaced with lexA operators, but remain transcriptionally inactive. As a next step, lexA-Aβ positive cells are transfected with a cDNA library suspected to encode binding partners of Aβ which are fusion proteins with 3 domains: a nuclear localization signal, a transcription activation domain, and an epitope tag. Upon proper binding of cDNA encoded protein to the lexA-Aβ fusion protein, the previously inactive lacZ and LEU2 reporters become transcriptionally active and permit the identification and isolation of cDNA which encode proteins that interact with Aβ. The characteristics of the clones isolated using this system will be discussed.

D3-311 **ASSOCIATION OF BOUND AUTOCHTHONOUS IgM ON T CELLS WITH STROKE AND ALZHEIMER'S DISEASE,** Kell SH, Allman RM, Harrell LE, Liu T, and Solvason N, University of Alabama at Birmingham and Veterans Affairs Medical Center, 933 19th St S, Birmingham, AL 35294-2041.

The binding of autochthonous IgM on the surface of CD3+ T cells has been described. To determine the medical correlates of this phenomenon, we studied adults with possible or probable Alzheimer's Disease (AD) by NINCDS/ADRDA criteria (n=24), stroke (n=15) and geriatric controls with other medical diagnoses (n=14). The 53 subjects had a mean age of 73.2 ± 9.1 years. AD patients had an increased number of IgM+ T cells per total T cells (%IgM+ T cells) compared with all other patients (35.6% ± 30.2% vs 14.6% ± 23.9%, $p<0.001$). In contrast, the stroke group had decreased %IgM+ T cells compared with all other patients (17.6% ± 31.9% vs 26.7% ± 27.3%, $p=0.02$). Within the stroke group, new strokes (those occurring <90 days before the study) had an even lower %IgM+ T cells than remote strokes (3.5% ± 4.1% vs 29.9% ± 40.6, $p < 0.05$). After excluding AD patients, new strokes remained associated with decreased %IgM+ T cells compared with the geriatric control patients ($p<0.05$). Future studies are needed to confirm this association with stroke and to clarify the mechanism(s) of the binding.

D3-312 YAC TRANSGENICS AND THE STUDY OF ALZHEIMER'S DISEASE, Bruce T. Lamb, Linda M. Call, Sekai R. Chideya and John D. Gearhart, Division of Developmental Genetics, Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, MD 21287

Several hypothetical mechanisms have been proposed for the generation and deposition of the amyloid beta (Aβ) peptide in Alzheimer's disease (AD). These include overexpression of the amyloid precursor protein (*APP*) gene, as suggested by Down Syndrome (DS, trisomy 21), and mutation of *APP*, as suggested by mutations associated with the presence of disease/amyloid deposition in some cases of familial AD (FAD). Although numerous *in vitro* studies have led to certain insights into the molecular basis for amyloid deposition, the mechanism(s) of amyloidogenesis *in vivo* remains poorly defined. To examine the role of APP in amyloidogenesis in an animal model, we have produced transgenic mice containing the entire 400 kilobase (kb) *APP* gene cloned in a yeast artificial chromosome (YAC) (Nature Genetics, **5**:22-30). This strategy has a major advantage over previous cDNA-based approaches in that the *APP* genomic sequences contain all the transcriptional regulatory elements required for proper spatial and temporal expression with appropriate splice donor and acceptor sites needed to generate the entire spectrum of alternatively spliced APP transcripts. *APP* YAC transgenics were produced by introduction of a 650 kb *APP* YAC into mouse embryonic stem (ES) cells by lipid-mediated transfection, and these cells were then used to generate mice retaining human *APP* sequences. These mice express human APP mRNA and protein at levels comparable to endogenous App, and are being examined for neuropathological, behavioral and neurochemical characteristics of AD some of which will be presented here.

To examine the effect of FAD mutations on amyloidogenesis *in vivo*, we have also focused on transferring *APP* YACs containing FAD mutations into ES cells and eventually into mice. Utilizing homologous recombination in yeast, we have generated YACs containing the 670/671 mutation (Lys/Met to Asn/Leu), the 717 mutation (Val to Ile), as well as both of these mutations together and have introduced these YACs into ES cells successfully. Finally, the ES cells are currently being used to generate mice with the mutated human *APP* gene. In conclusion, our YAC transgenic strategy should provide an accurate *in vivo* test for many of the hypothetical mechanisms of amyloidogenesis.

D3-313 **EFFECT OF TWO PATHOGENIC MUTATIONS ON THE CELLULAR PROCESSING OF THE MOUSE PRION PROTEIN.**

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Several mutations in the human prion protein (PrP) gene have been associated with inherited forms of prion disease. To see whether these mutations altered the cellular processing of PrP, we constructed a mouse PrP (moPrP) mutant (**E199K**) corresponding to a human mutation at codon 200 (E→K) that has been linked to familial Creutzfeldt-Jakob disease (CJD). We also constructed a mutant (**PG11**), which carries an insertion of 6 additional octapeptide repeats, and is equivalent to the 144 bp insertion seen in several CJD families.

We compared the processing of these two mutant proteins with that of wild-type moPrP in stably transfected CHO cells using a rabbit polyclonal antibody raised against the N-terminal part of moPrP. Using immunofluorescent staining, we found that while wild-type moPrP is expressed at the cell surface, **PG11** is retained intracellularly, largely in the perinuclear region. This mutation dramatically reduces the proportion of protein displayed on the cell surface, as confirmed by PIPLC treatment of the cells to separate surface from intracellular PrP. This defect results from reduced biosynthetic delivery of the protein to the surface, as demonstrated in pulse-chase labeling experiments. The molecule is also degraded at a significantly slower rate than the wild-type protein. We verified by PIPLC digestion that PG11 acquires a GPI anchor during its biosynthesis. We also demonstrated that PG11 is N-glycosylated, Endo H resistant and neuraminidase sensitive, suggesting that the molecule reaches the trans-golgi network. We are now attempting to identify the subcellular compartment(s) where the protein accumulates. The perinuclear distribution of the protein, and its posttranslational modifications, are most consistent with localization in the trans-golgi network, endosomes, or lysosomes. The **E199K** mutation results in aberrant glycosylation of the protein, which migrates on SDS-PAGE as a broad smear between 33 and 47 kDa, distinct from the more compact 40-45 kDa band seen with wild-type moPrP. That alteration in migration on SDS-PAGE reflects a difference in glycosylation is shown by the fact that the E199K and wild-type proteins comigrate after treatment with N-glycosidase F. This mutation does not block delivery of the protein to the cell surface, but the proportion of total protein on the surface seems to be reduced.

The differences in processing of PG11 and E199K described above may be relevant to the pathogenic effect of these mutations. The relationship between the altered trafficking of these mutants and formation of protease resistant and infectious isoforms of PrP are now being investigated.

D3-314 PD 114542 STAINS NEURITIC PROCESSES IN AFFECTED REGIONS OF ALZHEIMER'S DISEASE BRAIN BUT NOT IN AGE-MATCHED CONTROL BRAIN, Harry Levine, III, Jeff Scholten and Brenda D. Shivers, Department of Neurodegenerative and Neurological Disorders, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI 48106

Cryostat sections of freshly frozen Alzheimer's Disease (AD) brain tissue stained for five minutes with a 40 μM solution of PD 114542 in phosphate-buffered saline at room temperature displayed a bright network of fibers, tangles, and aggregates that fluoresce when viewed with the ex/em filter set for Thioflavin S. Brain areas involved in AD pathology such as the hippocampus and parietal cortex were stained, but the cerebellum containing diffuse plaques remains unstained. Neither rat brain nor sections from age-matched human brain sections from normal individuals were stained. This was the case for several aged normal and AD brains. Optical sectioning by laser confocal microscopy revealed that these structures, including plaque-like structures are solidly stained. Co-localization of PD 114542-staining structures with known epitopes were probed with a series of antisera as well as chemical and enzymatic treatments. The dye binding site tolerated aldehyde fixation and was partially sensitive to alkaline phosphatase treatment. The binding component remains unidentified as PD 114542-staining co-localizes with a subset of β/A4 plaques, neurofilaments and tau protein. We appreciate the gift of brain tissue from the University of Michigan AD Research Center and the Sun City Gerontology Institute.

D3-315 ZYME - A NOVEL AND POTENTIALLY AMYLOIDOGENIC ENZYME CDNA ISOLATED FROM ALZHEIMER'S DISEASE BRAIN, S. Little, E. Johnstone, E. Dixon, F. Norris, W. Buckly, G. Becker, M. Johnson, J. Dobbins, T. Wyrick, J. Miller, W. MacKellar, D. Hepburn, J. Corvalan, D. McClure, D. Stephenson and J. Clemens, CNS/GI/GU/Molecular Biology and Biotechnology Research Division, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285

A novel serine protease, given the name Zyme, has been identified from Alzheimer's disease (AD) brain. We have discovered this new serine protease by polymerase chain reaction (PCR) amplification of cDNA sequences representing active site homologous regions of chymotrypsin-like enzymes. A cDNA clone was identified which encodes a serine protease highly homologous to the known human serine protease, prostate specific antigen. Genomic DNA homologous to Zyme was detected in humans, primates, cats and dogs and was absent in mice, rats and hamsters. Zyme was found to be highly tissue specific. It was expressed predominantly in normal and AD adult brain, and was not detected in human fetal brain. The Zyme gene maps to chromosome 19 q13.3.

When Zyme cDNA was co-expressed with the Amyloid Precursor Protein (APP) cDNA in 293 (human embryonic kidney cells), amyloidogenic fragments were detected by Western blot analysis. A different pattern of cleavage products emerges when the 695 and 751 forms of APP are used. This novel protease cleaves full length APP as well as synthetic substrates and has a preference for cleavage after arginine (for example: residues -19, -24 and +5 of the beta amyloid peptide). Zyme can be immunolocalized to pericytes (perivascular cells) in primate brain tissue. Activated microglia cells are associated with AD pathology. Pericytes are thought to evolve into microglia cells. The potential role of Zyme in AD pathology will be discussed.

D3-316 TRANSGENIC ANIMAL MODELS FOR THE STUDY OF ALZHEIMER'S DISEASE. Jeanne F. Loring, Steve R. Schramm, Avidan Rose, Kathryn Wymore, Bruce Lenhart, Kay M. Higgins, Jim McCabe, Dennis Huszar, Chris Pászty[1], Edward Rubin[1], and Kenneth R. Peterson[2]. GenPharm International, Mountain View, CA 94043, [1]Human Genome Center, Life Sciences Division, Lawrence Berkeley Laboratory, Berkeley, CA 94720, and [2]Department of Medicine, Division of Medical Genetics, University of Washington, Seattle WA 98195.

A major obstacle to understanding the pathogenesis of Alzheimer's disease is the lack of easily studied animal models. We are applying transgenic methods to introduce human genes associated with AD into mice and rats. The use of large transgenes, although technically more difficult than using conventional transgenes, improves the level of transgene protein expression and the tissue specificity of expression. For example, the entire human *APP* gene (>400 kb) was introduced into mice as a 650 kb YAC (yeast artificial chromosome) transgene. The transgenic mouse we produced (Pearson and Choi, PNAS 90:10578) and a similar YAC transgenic mouse produced by Lamb and colleagues (Lamb et al, Nat Gen 5:22) both appear to express the human protein at about the same level as the endogenous protein (Buxbaum et al. BBRC 197:639), while levels of endogenous mouse APP seem to be unaffected. The presence of human APP (or the excess of total APP) does not have a profound effect on the development, behavior, or life span of the transgenic animals. The animals develop no spontaneous AD pathology, suggesting that simple overexpression of APP is not sufficient to produce an AD disease model. To ask whether overproduction of human βA4 peptide has any pathological effect in the rodent, we are modifying the APP YAC transgene to produce transgenic mouse and rat lines that carry a pathological mutation that causes early onset form of AD (Swedish mutation; Mullan et al, Nat Gen 1:345). In addition, we are producing mice and rats that carry the human apolipoprotein E4 variant, and are likely to express the human protein in astroglial cells. The apolipoprotein E4 variant is linked to late-onset AD and is thought to enhance development of senile plaques and/or neurofibrillary tangles in the aged brain (Roses et al. Science 261:921). These transgenic animals will be useful for dissecting the biochemical and physiological steps leading to AD, and for development of therapies for disease intervention.

D3-317 **ADENOVIRAL EXPRESSION OF THE AMYLOID PRECURSOR PROTEIN.** L. Mercken (1), L. Pradier (1), J. Clot (1), C. Marchal (1), E. Vigne (2), A.F. Macq (3), M. Perricaudet (2), J.N. Octave (3) & J.C. Blanchard (1).
(1) Molecular Neurobiol. Rhône-Poulenc Rorer S.A. Vitry-sur-Seine; France. (2) Institut Gustave Roussy; Villejuif; France. (3) UCL; Labor. Neurochem.; Brussels; Belgium.

The adenoviral expression system has been shown to be suitable for expression of recombinant proteins in quiescent cells, like neurons. In order to study the processing and to evaluate the toxicity of the amyloid precursor protein (APP) in neurons, an adenovirus was constructed with the cDNA of the 695 amino-acid isoform of the human APP under the control of the RSV-LTR promoter. Different cell lines and primary neuronal cultures were infected with the APP-adenovirus. Functionality of the adenovirus in these cell lines was evaluated by immunodetection of the APP in total proteins and in the culture medium by Western Blot analysis.

D3-318 DISTRIBUTION OF THE PRION PROTEIN AND ITS LIGANDS: IMPLICATIONS FOR SPONGIFORM ENCEPHALOPATHIES

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The infectious particle causing transmissible spongiform encephalopathies (denominated prion) is composed at least in part of the disease-specific prion protein PrP^{Sc}. PrP^{Sc} is derived from its normally occuring isoform PrP^{C}, probably by a change in conformation. We have reexamined the distribution of PrP producing cells, the prion protein itself and PrP binding sites on brain sections. Using *in situ* hybridisation and Northern blot analysis we were able to show that PrP mRNA is produced not only by neurons (as shown previously) but also by glial cells. This is especially important in view of the observed accumulation of PrP^{Sc} in white matter areas. It has been proposed that PrP^{Sc} is transported axonally and then deposited. Our results suggest that PrP^{Sc} is synthesized and accumulated locally by glial cells. The localization of the prion protein itself has previously been described in different brain areas predominantly grey matter. Using different immunohistochemical methods, we can now show that there may be different populations of PrP residing either in the vicinity of cell bodies or in axonal tracts. The latter is only revealed after acid/ethanol pretreatment of the tissue suggesting that it may be inaccessible under normal conditions. These results together with the previously oberserved accumulation of PrP^{Sc} in astrocytes argue that prion disease may have a glial origin. Last not least we describe binding sites present in normal or scrapie-infected brain sections. Binding sites were most prominent on pyramidal neurons and granule cells of the hippocampus and the granule cell layer of cerebellum. This distribution of binding sites argues for a function of PrP in axo-dendritic signalling in the hippocampus. The identity of the proteins present at the binding sites will be discussed.

D3-319 CELL TYPE-SPECIFIC EXPRESSION AND REGULATION OF THE CHONDROITIN SULFATE PROTEOGLYCAN FORM OF APP,

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Recently we showed that the chondroitin sulfate proteoglycan form of Alzheimer's amyloid precursor protein (APP-PG) is present in human and rat brains. In primary cell culture, the synthesis of this CSPG was confined to astrocytes. Neuronal or other glial cell types produced no APP-PG (1). Cell type-specific expression of APP-PG was also seen in transformed cell lines. C6 gliomas and N2a neuroblastomas were the only cell lines producing APP-PG among others we surveyed. This is in strong contrast to the CSPG form of the amyloid precursor-like protein 2 recently reported (2), which was produced by most cell types. Since the proportion of APP in the PG form varies with cell type and growth conditions, we were interested in determining the factors regulating production of the APP-PG in astrocytes. Our results showed that high cell density cultures produced relatively higher levels of APP-PG than low cell density cultures, suggesting that cell density affects production of this molecule. When astrocytes were grown in DMEM based media with defined supplements, little APP-PG production was observed. In contrast, astrocytes grown in Waymouth's defined medium produced higher levels of the APP-PG, even though the levels of the APP production were the same in both media, indicating that CSPG synthesis is regulated by factor(s) in the medium. Since involvement of CSPGs and astrocytes has been suggested in both neuroprotection and neuronal patterning, this APP-PG may play an important role in these functions.

1. Shioi *et al.*, 1994, Neurosci. Abst. 20:1642.
2. Thinakaran and Sisodia, 1994, J.B.C. 269:22099.

D3-320 EXPRESSION OF THE FAMILIAL FORM OF HUMAN APP VARIANT (V>I) IN TRANSGENIC MICE,

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Alzheimer's disease (AD) is a neurological disorder that disproportionately affects the population over 65 years of age. Afflicted individuals exhibit impaired cognitive function and memory. In Familial AD (FAD), mutations in the amyloid precursor protein (APP) specifically link the APP gene to an early onset of AD. We generated murine transgenic lines that show neuronal specific expression of the human APP751 FAD (V>I) isoform. The APP751 (V>I) isoform is specifically expressed in the brain of patients with early onset familial AD and may, therefore, represent a useful model. A unique distribution of the transgenic APP751 FAD mRNA was observed in the central nervous system with high levels of expression in the cortex, hippocampus, amygdala, the superficial layers of the superior colliculus and the central grey, by *in situ* hybridization. We show a high steady state level of expression of APP751 FAD protein by Western blotting at about 0.7 fold the level of the endogenous APP. Intraneuronal APP751 FAD protein aggregates visualized with antibodies directed against the N-terminal portion of the APP751 FAD protein can be seen only in the transgenic animals. Additional analysis is required to evaluate the role and significance of the intraneuronal APP751 FAD protein aggregates.

D3-321 CALPAIN-CALPASTATIN SYSTEM IN AGING AND IN ALZHEIMER'S DISEASE,

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It has been suggested that Alzheimer's Disease (AD) may result from defective protein catabolism. There is evidence that a disequilibrium between calpain, a calcium-activated neutral proteinase and its endogeneous inhibitor calpastatin may be a possible regulatory defective site. AD is associated to numerous biochemical changes in extraneuronal tissues, therefore it is reasonable to assume that peripheral cells may also be affected. For this reason we studied calpain-calpastatin system in erythrocytes from Alzheimer's patients in order to examine the presence of possible alterations associated with the disease. Furthermore we examined how this cellular system changes with age in normal subjects. The levels of calpain and calpastatin activity were found significantly increased in the old subjects in comparison to young subjects, while the values of calpain and calpastatin activity in erythrocytes of Alzheimer's patients were lower compared to those of normal subjects of same age.

Our results suggest that the calpain-calpastatin system in AD is not able to meet the increased requirements of age-related proteolytic activity, and this defect could allow abnormal accumulation and disarrangement of cytoskeletal elements (e.g. neurofilaments) that are calpain-substrates in neuronal cells.

D3-322 REGULATION OF APP SECRETION IN PRIMARY CULTURES OF RAT NEURONS AND GLIA, Katharyn Spiegel, Roshani B. Cowmeadow, and Charlotte Raby, Department of Neuroscience Therapeutics, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48106.Recent studies have suggested that pharmacological agents may increase the secretion of amyloid precursor protein (APP), thereby reducing production of ß-amyloid. However, these studies have utilized cultures of non-neuronal cells. To determine whether CNS production and secretion of APP may be pharmacologically controlled, we examined both cell associated and secreted APP in primary cultures of neurons and glia (primarily astrocytes) from rat hippocampus, cortex, basal forebrain and cerebellum. As shown previously for CHO cells and other non-neuronal cells, the secretion of APP by glia was increased by treatment of the cultures with carbachol, the phorbol ester phorbol myristate acetate (PMA) and the calcium ionophore A23187. There was a corresponding decrease in cell associated APP in glia with these treatments. In contrast, although strong constitutive secretion of APP was detected in the neuronal cultures, neither the secretion nor the cell associated levels of APP were affected by treatment with carbachol, PMA or A23187. Although the source of extracellularly deposited ß-amyloid in Alzheimer's disease is not known, these studies indicate the importance of examining APP regulation in cells native to the CNS, rather than peripheral non-neuronal cells. Further work will examine the regulation of ß-amyloid production in primary neuronal and glial cultures.

D3-323 **MECHANISMS UNDERLYING (CAG) EXPANSION IN HUNTINGTON DISEASE INCLUDE TISSUE SPECIFIC AND CIS-ACTING FACTORS**

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Huntington disease (HD) is associated with a trinucleotide repeat that is polymorphic in the general population, and expanded beyond the normal range on affected chromosomes. The underlying mechanism/s for the repeat instability and expansion are unknown, and may be similar in several other neuropsyciatric disorders caused by expanding trinucleotides.

We are studying the fidelity of repeat replication, using quantitative measurements of the level of CAG repeat length mosaicism, in different tissues from individuals affected with HD. The repeat shows highly tissue specific patterns of instability. For example, cerebral brain and sperm consistently display the highest levels of instability, whereas cerebellum and adrenal invariably have low levels of instability. No instability can be detected in tissues from normal controls, nor on the unaffected chromosome in HD patients, suggesting that the instability is associated with the underlying mutation and is influenced by tissue specific factors that could be developmentally regulated.

Although spermatogenesis is associated with a high level of repeat instability in almost every affected individual, there were significant differences between individuals. Surprisingly, the different levels of sperm CAG instability were not correlated with the basic CAG repeat size as seen in blood cells, but rather with the intergenerational *change* in allele size of each particular chromosome. For example, a chromosome that contracted in size from the affected parent to the proband, was associated with a very low level of repeat instability in the proband's sperm, whereas a CAG that had expanded in the previous generation was associated with a high level of instability in sperm. These correlations also extended to the following generation, such that a low instability in a proband's sperm was associated with repeat contraction in his offspring, and a high sperm CAG instability was associated with expansion in the offspring. These data strongly suggest the presence of one or more cis-acting factor/s affecting repeat instability, distinct from the CAG repeat length itself. Analyses of sperm from individuals with CAG repeat sizes near the affected range are now under way, in an attempt to provide a molecular basis for why some have offspring with expanded repeats, while others display stable transmission.

D3-324 BIOLOGICAL ROLES OF CHONDROITIN SULFATE MODIFICATION OF APLP2 AND APP IN CNS,

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Alzheimer's disease is characterized by the deposition of ß-amyloid, which is generated by proteolytic processing of amyloid precursor proteins (APP). Two amyloid precursor-like proteins APLP1 and APLP2 have been identified. Both APP and APLP2 mRNA undergo alternate splicing. We previously characterized chondroitin sulfate (CS) modification of APLP2-751 isoform, and now we show that insertion of a 12 amino acid sequence by an alternatively spliced exon disrupts CS modification of two of the APLP2 isoforms. In CNS, mRNA encoding CS-modified forms of APLP2 are highly represented in the olfactory epithelium, relative to mRNA encoding CS-free forms of APLP2. Immunocytochemical studies show that APLP2 is enriched in cilia of olfactory sensory neurons, and sensory axons and their terminals in glomeruli. Since olfactory sensory neurons regenerate and establish synaptic connections with their targets in the CNS, these results suggest a potential role for CS-modified APLP2 in axonal growth and synaptic connectivity.

In a manner similar to APLP2, alternative splicing of exon 15 regulates CS modification of APP. CS modification of wild-type or Swedish-variant of APP leads to a 50% decrease in the amount of ß-amyloid secreted from transfected COS-1 cells. CS modification, however did not affect the secretion of APP (PNII) and the p3 fragment. We are currently examining the relevance of this finding to metabolism of APP by various cell types in the CNS.

This work was supported by grants from NIA and the Adler Foundation.

D3-325 REGULATION OF THE MOUSE β-AMYLOID PRECURSOR-LIKE PROTEIN (APLP2) GENE: PROMOTER CHARACTERIZATION AND ANALYSIS OF MICE WITH TARGETED APLP2 ALLELES,

von Koch C.S.[1,3], Reed R.R.[1,2,4], Zheng H.[5] and Sisodia S.S.[3], Departments of Neuroscience[1], Molecular Biology and Genetics[2], The Neuropathology Laboratory[3], and the Howard Hughes Medical Institute[4], The Johns Hopkins University School of Medicine, Baltimore, MD 21205 and Merck Research Labs[5], Rahway, NJ

APLP2 is a member of a larger gene family that includes APP. Using S1 protection analysis, we detected several transcription initiation sites, with the predominant site located ~89 bp upstream of the translation start site. To examine the transcriptional regulation of APLP2, we have cloned and characterized ~2.7 kb upstream of the APLP2 transcription initiation site. We show that the APLP2 promoter lacks a typical TATA box, but contains a CAAT box, and several consensus sequences for putative transcription factors. To define promoter sequences required for APLP2 gene expression, we transfected hybrid plasmids with varying 5'-deletions of the APLP2 promoter fused to a CAT reporter gene into mouse neuroblastoma cells (N2a). Our studies reveal that 160 bp upstream of the predominant transcription start site are sufficient to direct high levels of CAT expression. In order to identify nuclear factors that bind to the APLP2 promoter, we performed mobility shift assays on fragments of the APLP2 promoter. We demonstrate that a 32 bp fragment (-425 to -456) binds nuclear factors from olfactory epithelium and brain, at non-overlapping sites, suggesting the presence of at least two different factors which may be tissue-specific.

In order to examine potential biological roles of APLP2 during development or aging, we are currently using gene targeting strategies to generate mice devoid of APLP2 expression. We have isolated independent ES cell lines of targeted APLP2 alleles and generated several chimeric animals. Analysis of mice with targeted APLP2 alleles in the germline will be presented.

D3-326 INHIBITION OF AMYLOID PRECURSOR PROTEIN EXPRESSION BY POTENTIAL ALZHEIMER'S DISEASE THERAPEUTICS CT3577 AND CT2583 . J.W. West, Y. Wang, and D. Leung. Department of Molecular Biology, Cell Therapeutics, Inc., Seattle, WA. 98119.

Overexpression or inappropriate processing of the amyloid precursor protein (APP), resulting in β amyloid peptide (βAP) deposition, is central to progression of Alzheimer's disease (AD). The inflammatory cytokines TNFα and IL-1 are elevated in AD brains and stimulate the expression of APP, suggesting that inflammation and inflammatory cytokines may play a role in the development of AD. We have screened a library of anti-inflammatory molecules, that inhibit TNFα and IL-1 signaling in a variety of biological systems, in a high throughput assay based on a DNA promoter for human APP linked to alkaline phosphatase as a reporter gene. CT3577 and CT2583 inhibit reporter gene expression with IC_{50}s below 1 μM. Inhibition of reporter gene activity correlates with inhibition of the expression of endogenous APP in cultured human neuroblastoma cells. Furthermore, CT3577 and CT2583 reduced basal expression levels of APP in cultured neuroblastoma cells with the same potency which they inhibited TNFα-induced APP expression. CT3577 and CT2583 represent two structurally distinct classes of compounds classified for their ability to inhibit the accumulation of specific species of phosphatidic acids (PA) normally seen in inflammatory or stress responses. Although western blot analysis showed inhibition of the expression of endogenous APP, northern blot analysis of total mRNA revealed no effect of CT3577 or CT2583 on expression of APP mRNA. Pulse chase experiments in cultured cells revealed that CT3577 and CT2583 are translational inhibitors of APP expression. CT3577 and CT2583 do not inhibit the expression of G3PDH suggesting some degree of specificity for translational inhibition by these compounds.

D3-327 GENERATION AND CHARACTERIZATION OF MICE WITH DISRUPTED AMYLOID PRECURSOR PROTEIN

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The β-amyloid peptide, the major component of the neuritic plaques characterizing Alzheimer disease (AD), is derived from a much larger amyloid precursor protein (APP). The APP is encoded by a gene of about 400 kb that undergoes alternative splicing to generate several isoforms, encoding proteins that range from 365 to 770 amino acid residues. Among these, APP695 is expressed predominantly in neurons and APP751 and APP770 can be detected in all the tissues examined. Mutations in the APP gene have been identified which cause familial, early onset Alzheimer's disease, providing strong evidence for the notion that APP metabolism is a central event in the progression of Alzheimer disease. APP is one of the most abundant proteins in the brain and the β-amyloid peptide is produced not only in AD patients, but also in cerebrospinal fluid (CSF) of normal individuals. To understand the *in vivo* function of APP and its metabolism, we have generated an APP null mutation in mice using the technology of homologous recombination in embryonic stem (ES) cells. We show here that the APP message and protein are completely absent in the homozygous mice. However, the APP deficient mice were produced at expected frequencies. The APP null mice are fertile and do not show overt abnormalities at up to 14 weeks of age. A detailed pathology study will be discussed. The APP deficient mice should serve as a valuable strain to insert the human APP gene in an effort to generate a mouse model of Alzheimer's disease.

Trophic Factors as Potential Therapeutic Agents; Cellular Grafts and Delivery

D3-400 GDNF PROTECTS MESENCEPHALIC DOPAMINERGIC NEURONS AGAINST AXOTOMY INDUCED DEGENERATION IN ADULT RATS, Klaus D. Beck, Janet Valverde, Tajrena Alexi*, Arnon Rosenthal, Kris Poulsen, Heidi Philipps, Mark Armanini, Barbara Moffat, and Franz Hefti, Department of Neuroscience, Genentech, Inc., South San Francisco, CA 94080, and *Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089

GDNF, a recently cloned distant member of the transforming growth factor beta superfamily, is a potent survival factor for embryonic dopaminergic neurons *in vitro*. The regional expression of GDNF mRNA during development suggests a role as transient target-derived trophic factor for dopaminergic neurons in the substantia nigra of the mesencephalon. We used a rat model of lesion-induced adult dopaminergic neuron atrophy to test whether GDNF has trophic effects on dopaminergic neurons in the adult brain. Defined transection of the medial forebrain bundle which contains ascending axons of the dopaminergic neurons resulted in the loss of half of the population of tyrosine hydroxylase immunopositive neurons in the substantia nigra. This loss was largely prevented by repeated injections of GDNF in the vicinity of the substantia nigra. TGFalpha and NT-4/5 did not protect lesioned dopaminergic neurons. Our findings represent the first characterization of a trophic factor protecting the dopaminergic phenotype of substantia nigra neurons in the adult brain and suggest the use of GDNF in Parkinson's disease which is characterized by the degeneration of these cells.

D3-401 *EX VIVO* GENE THERAPY WITH BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) IMPROVES BEHAVIOR IN A RAT MODEL OF PARKINSON'S DISEASE, Martha C. Bohn, Yusuke Yoshimoto, Qing Lin, Timothy J. Collier, David M. Frim and Xandra O. Breakefield, Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14624 and Molecular Neurogenetics Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114.

Previous studies have identified BDNF as a neurotrophic and neuritotrophic factor for embryonic dopaminergic (DA) neurons in culture. To determine whether BDNF might have similar effects on DA neurons in the adult brain, we have used an *ex vivo* gene therapy approach to deliver biologically produced BDNF into the rat striatum following a partial lesion of the DA projection with 6-hydroxydopamine (6-OHDA). Astrocytes were purified from the neonatal rat cortex and infected with a replication deficient retrovirus harboring human preproBDNF cDNA under control of the Moloney leukemia virus LTR and a selectable gene, neomycin phosphotransferase. Following selection in G418, the astrocytes were injected into 4 sites in the striatum (20,000 cells/site) 2 weeks after an injection of 6-OHDA into the lateral substantia nigra. Rats grafted with astrocytes infected with a retrovirus harboring alkaline phosphatase (AP) served as controls. At 2 and 4 weeks after grafting, amphetamine-induced rotational behavior was reduced in the BDNF, but not AP group. However, image analysis of the density of DA fibers in the striatum did not reveal a significant difference between BDNF and control groups. These observations suggest that increased levels of BDNF in the adult striatum may stimulate function of the DA system through a mechanism other than stimulating regeneration or sprouting of DA fibers that remain after a partial 6-OHDA lesion.

D3-402 USE OF RETROVIRALLY TRANSDUCED ASTROCYTES TO DELIVER RECOMBINANT BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) TO MIDBRAIN DOPAMINE NEURONS IN CULTURE: IMPLICATIONS FOR GENE THERAPY IN PARKINSON'S DISEASE, L.A. Cunningham, I. Lopez-Colberg, K. Krobert, X.O. Breakefield[1] and D. Frim[1], Dept. Pharmacology, Univ. New Mexico Health Sciences Center, Albuquerque, NM 87131 and [1]Molecular Neurogenetics Unit, MGH, Harvard Medical School, Boston MA 02114.

Previous studies demonstrated that retrovirally transduced astrocytes can be used to deliver recombinant nerve growth factor (NGF) to the brain (Cunningham et al., Brain Res., *in press*). To determine whether astrocytes can be used to deliver a neurotrophic factor for midbrain dopamine neurons, type I astroctyes purified from newborn rat striatum were infected with a MoMLV-based replication defective retrovirus harboring the human prepro BDNF gene sequence under LTR transcriptional control. Neuronal cultures established from embryonic day 15 rat ventral mesencephalon were maintained for six days in normal N2 medium, medium conditioned by astrocytes infected with the BDNF retrovirus (AsBDNF-CM), or medium conditioned by astrocytes infected with a control retrovirus harboring the NGF cDNA sequence (AsNGF-CM). Exposure to AsBDNF-CM stimulated a 4-7-fold increase in the number of tyrosine hydroxylase-immunoreactive (TH+) neurons compared with controls ($p<0.01$). When the neuronal cultures were exposed to 100 ng/ml purified BDNF protein, there was a 2.5-fold increase in the survival of TH+ neurons. These studies suggest that primary astrocytes transduced with a BDNF-containing retrovirus produce biologically active BDNF, which promotes the survival of dopamine neurons in culture. Transplantation studies designed to test whether astrocytes can also be used to deliver BDNF to affect dopaminergic neurons in a rat model of Parkinson's disease are currently underway. Supported by NINDS NS32562

D3-403 CROSSREACTIVITY OF NEUROTROPHINS VIA THE LOW AFFINITY RECEPTOR $p75^{NGFR}$ C. Hertel and A. Müller, Pharma Division, Preclinical Research, F. Hoffmann-LaRoche AG, 4002 Basel, Switzerland

The functional interaction of the low affinity nerve growth factor receptor $p75^{NGFR}$ and the tyrosine kinase receptor trkA is still unresolved. All neurotrophins bind to $p75^{NGFR}$, while the tyrosine kinase receptors are selective for neurotrophins, e.g. TrkA binds NGF and with much lower affinity NT3 and NT4/5, but not BDNF. The functional response is mediated by trk dimerization which induces a phosphorylation cascade that ultimately promotes survival and differentiation. The role of $p75^{NGFR}$ is still unclear. Recent results from several laboratories suggest that $p75^{NGFR}$ may act to increase the local effective concentration of NGF for trkA, thereby increasing the cellular response at low concentrations. To investigate the functional interaction of $p75^{NGFR}$ with TrkA, a glial cell expressing $p75^{NGFR}$, but neither TrkA nor TrkB, were transfected with TrkA. TrkA transfected 33Bcells, 33B-6, express high and low affinity binding for TrkA. Stimulation of 33B-6 cells results in phosphorylation of TrkA and phospholipase $C\gamma$, stimulates TrkA expression, and induces formation of cellular processes, while wt 33B cells do not respond. Concomitant treatment with BDNF and NGF reduced binding of NGF not only to $p75^{NGFR}$ but also to TrkA. Thus the presence of BDNF not only effects signalling by its cognate receptor TrkB, but also alters the signalling of NGF by competing for the common $p75^{NGFR}$ receptor and thereby reducing the local concentration of NGF for TrkA.

D3-404 **BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF)-SECRETING GRAFTS PROTECT DOPAMINERGIC NEURONS *IN VIVO* FROM PARTIAL 6-HYDROXYDOPAMINE (6-OHDA) LESIONS,** Un Jung Kang, Marc Levivier, Craig Bencsics and Serge Przedborski, Dpts of Neurology and Pharm/Physiol., Univ. of Chicago, Chicago, IL 60637 and Dpt. of Neurology, Columbia University, New York, NY 10032

OBJECTIVE: To develop a delivery system for BDNF into the central nervous system (CNS) by using genetically modified cells and to test its neuroprotective effect in a rat model of early stages of Parkinson's disease (PD). **BACKGROUND**: BDNF has neuroprotective properties on DA neurons *in vitro*. However, BDNF is difficult to deliver into the CNS and therefore its *in vivo* effects are not well-defined. We have shown that BDNF-secreting fibroblasts can induce neurite sprouting of adult dopaminergic neurons *in vivo* (Lucidi-Phillipi et al., J Comp Neurol, in press). To test its neuroprotective effect, we developed a model of partial lesion of the nigrostriatal DA pathway using injection of 6-hydroxydopamine into the caudate-putamen complex (CPu). This lesion paradigm results in a dose-dependent reduction in [3H]mazindol-labeled DA uptake sites at the level of the CPu and SNpc; the later correlates with SNpc cell count. Striatal neurons were unaffected by 6-OHDA as suggested binding for D1, D2 and adenosine A2 receptors (Przedborski et al., Neuroscience, in press). **DESIGN/METHODS**: Primary skin fibroblasts were genetically modified with a retroviral vector expressing human BDNF and implanted in to the CPu of syngeneic rats 2 weeks prior to the 6-OHDA injection. Controls rats received unmodified fibroblasts. Partial lesions of the nigrostriatal DA pathway were induced by injecting 6-OHDA into the CPu. Lesions were assessed by binding autoradiography and cell count on Nissl staining at the level of the SNpc and by binding autoradiography at the level of the CPu. **RESULTS**: Compared to animals grafted with unmodified fibroblasts, rats grafted with BDNF-secreting fibroblasts were protected against 6-OHDA-induced neurodegeneration, as evidenced by [3H]mazindol binding and SNpc cell count. **CONCLUSION**: Grafts of primary skin fibroblasts genetically modified to secrete BDNF protect adult dopamine neurons against partial 6-OHDA lesions delivered to the CPu. These results open new possible neuroprotective therapeutic avenues aiming at slowing down neurodegeneration in early stages of PD, using neurotrophic factors such as BDNF.

D3-405 INCREASED α1-ANTICHYMOTRYPSIN IN CSF AND SERA FROM PATIENTS WITH PROBABLE ALZHEIMER'S DISEASE ASSESSED BY ELISA AND WESTERN BLOT.
Federico Licastro, Valentina Sirri, M. Cristina Morini, L. Jane Davis, Davide Trerè, Domenico Cucinotta. Dept. of Exp. Pathol. Univ. of Bologna and Dept. of Geriatrics, USL 27, Bologna, I-Italy.
The ß-amyloid protein (ß-AP) is the main component of the proteinaceous deposits in brain lesions of AD patients. Other proteins, such as protease inhibitors and complement components have been found in amyloid deposits. In particular, a serine protease inhibitor called α_1-antichymotrypsin (α_1-ACT) is commonly found associated with ß-AP in brain lesions. At the present, contradictory findings regarding the possible presence of elevated levels of α_1-ACT in CSF and sera from patients with AD are on record. Our previous experience indicated that increased levels of this serpin were specifically associated with AD.
Here we measured the concentration of α_1-ACT in CSF and sera from patients with probable AD, vascular dementia and from non demented elderly using a competitive immune enzyme assay (ELISA) or a Western blot with subsequent quantitation by computerized densitometry. According to ELISA assay CSF levels of α_1-ACT from AD patients were higher than those from VD patients and controls. Western blot appeared to be less sensitive than ELISA but a difference was still present between AD and VD patients or controls. It is of interest that by the latter assay a high molecular form (≈ 160 Kd) of α_1-ACT was detectable in some patients with AD. The ELISA assay revealed that serum levels of α_1-ACT were increased in a group of patients with AD. Western blot showed that the high molecular form of this serpin was the one increased in sera from patients with probable AD.

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D3-406 CHICKEN PERIPHERAL NEURONS : A SYSTEM TO PROBE THE BRAIN-DERIVED NEUROTROPHIC FACTOR STRUCTURE-FUNCTION RELATIONSHIPS AND THE MOLECULAR MECHANISMS OF APOPTOSIS.
Annick Martin, Esther Toselli, Charles Auffray and Marie-Dominique Devignes, Génétique Moléculaire et Biologie du Développement, CNRS-UPR 420, 7 rue Guy Môquet, F 94801 Villejuif cedex, France.
The primary culture of chicken embryonnary neurons constitutes a cheap and flexible system for the study of neurotrophic factors and neuronal death. We have adapted the culture of sympathetic, nodose and dorsal root neurons to a 96-well microtitration plate assay aimed at measuring the neurotrophic activities of Nerve Growth Factor (NGF) and Brain-Derived Neurotrophic Factor (BDNF), both members of the neurotrophin family. Several parameters vary with the source of neurons to be used such as the developmental stage (E8 - E11) and the number of days in culture. The assay involves a quantitative measurement of neuron survival using the mitochondrial transformation of a yellow tetrazolium ion (MTT) into a blue formazan compound (Manthrope et al. 1986, Dev. Brain Res 25: 191-198).
The understanding of neurotrophin specificity of action as well as the design of neurotrophin-based drugs involve a good knowledge of the structure-function relationships among neurotrophins. We have undertaken such a study with mouse BDNF. Mutations at positions strictly conserved across species are introduced in the sequence using a strategy based on homologous recombination. Expression in COS cells allows the secretion of native or mutated versions of BDNF in culture supernatants which can be assayed in the survival test described above. Other tests are envisaged to characterize the biological effects of each mutations.
In parallel, we took advantage of the availability of the survival assay to investigate *in situ* some of the molecular mechanisms of neuronal apoptosis induced by NGF withdrawal. To this aim, we optimized the time of addition of the anti-NGF antibody in the culture. Results concern in particular early mitochondrial events and nuclear DNA fragmentation.

D3-407 HERPES SIMPLEX VECTORS FOR VIRUS-MEDIATED GENE SUPPRESSION IN THE CNS,
Christopher A. Meaney and Joseph C. Glorioso: Department of Molecular Genetics and Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.
Herpes simplex virus (HSV) derived vectors provide a method for the expression of manipulated genetic material within post-mitotic neuronal cells and may prove useful both in treatment of CNS disorders and for basic studies of nervous system function. Because the high transcript levels produced by RNA polymerase III may be useful for antisense or ribozyme suppression of mutant proteins in some forms of gene therapy plasmids containing the VA_I gene of Adenovirus were integrated into replication-defective and replication attenuated HSV backbones using a phage (CRE/*lox*) recombination system. The VA_I RNA inhibits shutoff of cellular protein synthesis mediated by the interferon and dsRNA activated protein kinase DAI (Mathews and Shenk 1991). Northern and primer extension analysis of total cellular RNAs revealed that VA_I expression is induced both by viral immediate early gene expression and by viral replication. Transcription of VA_I was also found to occur independently of viral gene products in cells which were pretreated with the inhibitor of protein synthesis anisomycin. We have examined HSV vectors where the VA_I promoter expresses a full length antisense RNA of the mouse *c-fos* first intron and exon and an RNA terminating prematurely within the intron. *c-fos* mRNA induced in PC12 cells with dbcAMP is degraded by this virus while tyrosine hydroxylase mRNA is not affected. Both mRNAs are unaffected when induced during control infections. Insertion of *fos* sequences into VA_I RNA also decreases transcript half life from greater than 11 hours for VA_I to less than 30 minutes for the hybrid. We are presently examining the effect of these viruses on the mRNAs and proteins of related bZIP family members and transcription of their potential targets. Because both DAI kinase action and *c-fos* protein have been shown to induce apoptosis in cultured cells we plan to examine the ability of these viruses to suppress this process.

D3-408 EMBRYONIC STRIATAL TRANSPLANTS INTO QUINOLINIC ACID-LESIONED RAT STRIATUM AMELIORATE HOST GLIAL RESPONSES
A. Jennifer Morton and Lawrence M. Tucker, Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ, United Kingdom.
Rats with quinolinic acid (QA) striatal lesions are commonly used as animal models for Huntington's disease (HD). The characteristics of the neuronal loss and host glial reactions of QA-lesioned striatum suggest that they are good models for the later stage of the disease, and therefore resemble the brains of patients who might be considered for therapeutic neuronal transplants.
We examined the striata of QA-lesioned rats over a period of three months, and found that the glial reaction to the lesion was dynamic over this period, with profound changes in populations of astrocytes, macrophages and microglia. When we transplanted embryonic striatal neurons into QA-damaged host brain we found that the glial reaction normally seen was markedly reduced. In particular, the macrophages and microglia which are usually a significant component of the host reaction were excluded from the grafts and from a region surrounding the transplants beyond the host/graft border.
In HD, where the underlying pathogenesis of the disease is not understood and the neuronal loss is not restricted to a single class of neurons, the logic behind neurotransplantation may seem somewhat lacking. However, it is becoming increasingly clear that glial cells are not just innocent bystanders in HD, but that they may actively contribute to the neurodegenerative process. If this is so, then the prevention or reduction of the malevolent influence of glial cells by embryonic transplants may be critically important to the therapeutic potential of neural transplantation in HD.

D3-409 CHARACTERIZATION OF FGF-RESPONSIVE PROGENITOR CELLS ISOLATED FROM ADULT RAT HIPPOCAMPUS, SEPTUM, STRIATUM, AND STRIATAL SUBVENTRICULAR ZONE, T.D. Palmer, J. Ray, H.K. Raymon, H.G. Kuhn, D.A. Peterson, J. Suhonen, L.J. Fisher, and F. H. Gage. The Salk Institute, La Jolla, CA 92093

Genetic models for the treatment of neurodegenerative disease focus on delivering genes into postmitotic neurons *in situ* or on moderating a disease phenotype by grafting engineered non-neuronal cells. Until recently, fetal tissues were considered the only source of proliferative neuronal progenitors suitable for replacement grafts, however, mounting evidence suggests that immature neural progenitors reside in several regions of the adult mammalian brain. We have observed that cells with characteristics of both glial and neuronal progenitors can be recovered from the adult rat hippocampus, septum, substantia nigra, striatum, and striatal subventricular zone using defined medium supplemented with basic fibroblast growth factor. *In vitro*, many cells express markers characteristic of both immature oligodendroglial and neuronal lineages. These markers include A2B5, O4, Nestin, NSE, Map2, Map5, GAD, Calbindin, and others. When cultured hippocampal progenitors are genetically marked and grafted to the adult rat striatum or hippocampus, many of the marked cells differentiate into astrocytes or oligodendrocytes, however, some cells show characteristic markers and morphology of neurons. The presence of marked neurons and glia within the grafts demonstrates that the adult rodent brain contains immature cells with the potential to proliferate and reconstitute both neuronal and glial populations. If similar cells exist within the adult human brain, appropriate mitogenic signals might allow autologous tissues to be stimulated *in vitro* or *in situ* to proliferate and replenish neural populations lost due to injury or degenerative disease.

D3-410 GLYCATION INCREASES THE PERMEABILITY OF PROTEINS AT THE BLOOD NERVE AND BLOOD BRAIN BARRIERS, Joseph F. Poduslo, Geoffry L. Curran, Molecular Neurobiology Lab, Mayo Clinic, Rochester, MN 55905.

We have demonstrated increased permeability at the blood nerve barrier (BNB) of albumin after glycation with D-glucose (*PNAS* 89 (1992) 2218). The generality of this observation was evaluated by measuring the permeability coefficient-surface area product (PS) after correction for the residual plasma volume (V_p) at the BNB, as well as the blood brain barrier (BBB), for nerve growth factor (NGF) and human IgG after *in vitro* glycation with D-glucose, using an i.v. bolus injection technique in the cannulated brachial vein and artery of normal adult rats. The PS of the BNB obtained for gNGF was significantly increased compared to NGF with a 2.0 fold increase observed after 8 weeks of glycation and a 5.1 fold increase at 21 weeks of glycation. The V_p measurement for NGF and gNGF at the BNB was not significantly different at 8 weeks of glycation but was 1.3 fold greater at 21 weeks of glycation. The PS at the BBB for gNGF was 2 fold greater than NGF with a glycation time of 8 weeks and 3.2-3.6 fold greater with a glycation time of 21 weeks for six different brain regions. No changes were observed in the V_p for any of the brain regions for gNGF compared to NGF. The PS at the BNB for gIgG compared to IgG was significantly greater with a 4.1 fold relative increase. The PS at the BBB for gIgG ranged from a 2.8 fold increase for the thalamus to a 5.1 fold increase for the caudate putamen when compared to IgG. No significant differences were observed for the V_p values. These data demonstrate that glycation can enhance the permeability at the BNB and BBB of proteins with widely varying M_r and function. Since the glycation of NGF does not appear to affect its neurotrophic activity, systemic delivery of gNGF might be useful for treating a variety of neurodegenerative diseases. Similarly, the glycation of immunoglobulins might be a convenient procedure for delivery of a variety of antigens into the nervous system. NS14304

D3-411 CELLS CULTURED FROM THE ADULT SUBSTANTIA NIGRA SURVIVE AND DIFFERENTIATE FOLLOWING GRAFTING INTO ADULT RAT BRAIN. H.K. Raymon, T.D. Palmer, J. Ray and F.H. Gage, The Salk Institute, La Jolla, CA 92093

The presence of proliferative neural progenitor cells in the adult has been suggested by *in vivo* labeling studies utilizing markers of cellular proliferation. Isolation of neural progenitor cells from the subventricular zone of the striatum has been achieved *in vitro* in the presence of epidermal growth factor. Cell cultures of these progenitors give rise to both neurons and glial cells. Using the potent mitogen basic fibroblast growth factor (FGF-2), we have recently isolated neural progenitors from a number of regions in the adult brain. We report here that cell cultures from the adult rat substantia nigra (SN) can yield a proliferative population of neural progenitors that give rise to both neuronal and noneuronal cell types when isolated in the presence of FGF-2. Transplantation experiments were conducted in order to determine the influence of local adult cues on the fate of SN neural progenitors. Prior to transplantation into the striatum, cells were labeled both with bromodeoxyuridine (BrDU), which incorporates into dividing cells and with an adenoviral vector carrying the reporter gene, β-galactosidase (β-gal). SN neural progenitor cell cultures survive grafting into the intact striatum for up to 8 weeks as evidenced by both positive staining with antibodies recognizing BrDU and β-gal. The morphology of the grafted cells resembled that of both neuronal and nonneuronal cells. Double-label immunocytochemistry for BrDU and either NeuN, a neuronal marker or GFAP, a glial cell marker, indicated that some of the graft-derived cells were in fact neurons and glial cells. Future experiments will further examine factors or signals that influence the fate of these neural progenitors both *in vitro* and *in vivo*.

D3-412 ANALYSIS OF NGF MUTANTS WITH A NOVEL, HIGH CAPACITY, QUANTITATIVE KINASE RECEPTOR ACTIVATION (KIRA) ELISA. Michael D. Sadick*, John Winslow^, David Shelton^, Marcel Reichert*, Gary Laramee^ and Wai Lee T. Wong*. Departments of *BioAnalytical Technology/Research Immunochemistry and ^Neurobiology, Genentech, Inc, South San Francisco, CA, 94080.

A rapid, sensitive, and high capacity assay has been developed to quantify ligand-induced receptor tyrosine kinase activation in terms of receptor-autophosphorylation. The assay, termed a "Kinase Receptor Activation" or KIRA-ELISA, consists of two separate microtiter plates, one for cell culture and ligand stimulation, and the other plate for receptor capture and phosphotyrosine ELISA. The assay was developed for analysis of neurotrophin-induced trkA, trkB or trkC activation. It utilizes a trkA, trkB or trkC receptor fused with a 26 amino acid polypeptide flag derived from HSV glycoprotein D (gD.trkA,B or C, respectively) on the amino-terminal end, stably transfectanted into CHO cells. Stimulated receptors were solubilized with Triton X-100 buffer and then captured in ELISA wells coated with gD-specific mAb. The degree of receptor autophosphorylation was quantified by anti-phosphotyrosine ELISA. Reproducible standard curves were generated with a EC_{50} of approximately 528 pM NGF for gD.trkA KIRA, 139 pM for NT4/5 and 516 pM for NT3 in gD.trkB KIRA, and 143 pM for NT3 in gD.trkC KIRA. The gD.trkA KIRA was used to analyze the ability of NGF mutants to activate trkA. For seven different mutations studied, KIRA results were compared to those of PC12 neurite outgrowth assays, trkA binding assays and p75 binding assays. There was good correlation between KIRA-ELISA results and those of the other assays. All mutations had measurable effect on PC12 outgrowth (a decrease in EC_{50} from 20% to 98 % compared to wt NGF), although only 3 of 7 had an effect on maximum value. Only 1 mutation had an effect on p75 binding (R69A), while all mutations decreased trkA binding. Lastly, all mutations decreased both maximum gD.trkA autophosphorylation and, except for one mutant (Y79A + T81A), NGF/gD.trkA EC_{50} values. The data demonstrate that the KIRA-ELISA is a powerful and effective assay to assess bioactivity of putative ligands and ligand mutants for receptor activation.

D3-413 **A K252A ANALOG PREVENTS LOSS OF CHOLINERGIC NEUROCHEMICAL AND HISTOLOGICAL PARAMETERS IN RATS SUBJECTED TO LESIONING OF THE NUCLEUS BASALIS MAGNOCELLULARIS.** M.S. Saporito, F. Haun, E.M. Brown, M.A. Glicksman, M.E. Forbes, J.E. Prantner, A. DiCamillo, C. Murakata*, N. Neff and S. Carswell. Cephalon Inc., 145 Brandywine Parkway, West Chester, PA 19380 and *Kyowa-Hakko, Tokyo, Japan.

Loss of basal forebrain cholinergic neurons is a primary deficit associated with Alzheimer's disease (AD). In experimental animals, lesioning of analogous cholinergic neurons results in loss of cholinergic markers in terminal projection fields and cell bodies,as well as deficits in learning and memory. Nerve growth factor (NGF) increases cholinergic function in these models and, on this basis, is proposed as a therapeutic for neurodegenerative disorders including AD. However, NGF's therapeutic potential is limited by its inability to penetrate the blood-brain barrier. Alternatively, small organic molecules that have neurotrophic activities and which can penetrate into the brain may be effective in these disorders. K252a is such a molecule and has been shown to promote neuronal survival in a variety of primary neuronal cell culture systems. A systematic evaluation of K252a analogs revealed a compound, CEP1347, which was more potent and efficacious than the parent compound with respect to increasing ChAT activity in basal forebrain neurons in culture. Thus, CEP1347 was assessed for its ability to affect compromised cholinergic neurons *in vivo*. Unilateral lesioning of the nucleus basalis magnocellularis (nBM) with ibotenic acid elicited a regionally selective 30% decrease in cortical ChAT activity, a 50% loss of cortical AChE staining and a concomitant decrease in ChAT immunoreactive cell bodies in the nBM. Systemic administration of CEP1347, beginning either before or the day after lesioning, attenuated the decrease of all cortical and nBM cholinergic parameters at 4, 14 and 21 days after the lesion. In unilaterally lesioned rats, contralateral cholinergic parameters were unchanged, suggesting that CEP1347 was affecting only lesioned neurons. The effects of CEP1347 contrasted with the effects of NGF. NGF, infused ICV, increased cortical ChAT activity 14, but not 4 days after lesion. Furthermore, NGF had no effect on AChE stained cortical neurites and, unlike CEP1347, increased the size of ChAT immunoreactive cell bodies in the nBM. These results indicate that CEP1347 is neurotrophic for cholinergic neurons *in vivo* in a way that is distinct from NGF. Furthermore, these data suggest that the K252a class of molecules may be useful in neurodegenerative disorders involving loss of basal forebrain cholinergic neurons.

D3-414 THE INHIBITION OF MICROGLIAL ACTIVATION BY A NOVEL ANTI-INFLAMMATORY COMPOUND. Brenda D. Shivers, Jennifer L. Grimm, Richard D. Dyer, Karen M. Keane and Richard T. Carroll, Neurological and Neurodegenerative Diseases Group, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105

Microglia (brain macrophages) have been implicated in a variety of neurodegenerative diseases. In a resting state, microglia play a role in the housekeeping of the brain. When activated, microglia produce cytokines which are lethal to neurons. Preventing the activation of peripheral macrophage has been successful with the use of cyclooxygenase (COX) inhibitors such as indomethacin and ibuprofen. However, the use of COX inhibitors in neurodegenerative diseases has not been thoroughly investigated. To study the effects of arachidonic acid metabolites on activated microglia we used the microglia cell line (BV-2) and activated it with lipopolysaccharide (LPS). To follow activation we measured nitric oxide synthase (NOS) activity, which is induced by cytokines, and APP secretion. LPS-treated microglia produced detectable levels of nitrite in the media 8 hours after activation. The production of nitrite was inhibited with N^G-methylarginine, a specific inhibitor of NOS. A 5-fold increase in APP secretion was also detected within 4 hours of activation. When the microglia were pretreated with indomethacin prior to LPS activation, no decrease in NOS activity or APP secretion was detected. Also, inhibition of PLA_2 with either manoalide or scalaradiol had no effect on LPS-stimulated NOS activity or APP secretion. These data indicate that arachidonic acid metabolites are not involved in the LPS induction of NOS or APP secretion. However, we have identified an anti-inflammatory compound (CI-987) that does inhibit both the induction of NOS activity and the increased secretion of APP with an IC_{50} of 2.4 μM. The characterization of this compound will also be presented.

D3-415 **INVOLVEMENT OF REK7, AN EPH-RELATED TYROSINE KINASE RECEPTOR, IN AXON BUNDLE FORMATION IN CULTURED CORTICAL NEURONS** Janet Valverde, Klaus D. Beck, John W. Winslow, Paul Moran, Ai Shih, Ingrid W. Caras and Franz Hefti; Department of Neuroscience, Genentech, Inc., South San Francisco, CA 94080

REK7 is an Eph-related tyrosine kinase receptor expressed in hippocampus and cortex. The expression of REK7 mRNA increases in late development and remains high in the adult brain. We used a soluble recombinant fusion protein (REK-IgG), consisting of REK7 extracellular domain and the Fc fragment of human IgG to analyze the biological role of this receptor. REK-IgG prevented the formation of axon bundles in co-cultures of purified cortical astrocytes and cortical neurons. In these cultures, differentiation of neurons reaches an advanced stage analogous to late development in the brain, when neural pathways and tracts are laid down. One manifestation of this is the formation of axon bundles which can be immunostained with antibodies against tau, but not with antibodies against MAP-2, a dendritic protein. The absence of axon bundles in REK-IgG treated co-cultures suggests a role for REK7 in the process of axon fasciculation, a crucial step in nervous system development and in the regeneration from injury or neurodegenerative lesions.

Late Abstracts

PROCESSING OF AMYLOID PRECURSOR PROTEIN IN CHINESE HAMSTER OVARY CELLS IS SIMILAR IN 670/671 MUTANT AND WILD TYPE APP EXPRESSING CELLS, Ruth G. Perez and Edward H. Koo, Center for Neurologic Diseases, Department of Neurology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115 A mutation at codon 670/671 of ßPP (KM - NL) from a Swedish kindred with familial Alzheimer's disease generates elevated Aß secretion. Because we have demonstrated that Aß production and release involves the internalization and recycling of cell surface ßPP, we evaluated codon 670/671 mutation effects on ßPP processing in this pathway. As expected, Aß release was significantly elevated in CHO cells transfected with the KM - NL mutation (**sw**) as compared to ßPP751 (**wt**) cells. By metabolic labeling with a short pulse of ^{35}S-methionine, the kinetics of Aß release was essentially the same for **wt** and **sw** cells, with only the amount of Aß secretion varying. The enhanced secretion of Aß in **sw** cells correlated with increased 12 kDa C-terminal ßPP fragment. In addition, a lower molecular weight ßPPs species compatible with "ß-secretase" cleaved ßPPs was present intracellularly and was released into the medium of **sw** cells. Abundant intracellular cleavage of ßPP by ß-secretase decreased the amount of full length ßPP sorted to the cell surface in **sw** cells. However, Aß secretion was still elevated in **sw** cells after cell surface iodination, demonstrating that even with fewer full length ßPP molecules they still produce more Aß from the surface-labeled ßPP molecules. Both **sw** and **wt** cells showed similar responses to various drugs which alter vesicular pH. Our results indicate that the 670/671 mutation produced a selective alteration of ßPP processing by ß-secretase and that the **sw** cells produce more Aß in both the secretory and endocytic pathways.

DEVELOPMENTAL FATE OF SENSORY NEURONS IN TRKA AND TRKC KNOCKOUT MICE. [1]Inmaculada Silos-Santiago, [2]Fletcher A. White, [2]Shigeru Ozaki, [2]William D. Snider, [1]Mariano Barbacid. [1]Dept. Molecular Biology, Bristol-Myers Squibb PRI, Princeton, NJ, 08543. 2Dept. Neurology, CSNSI, Washington Univ. Med. Sch. St. Louis, MO 63110.

We have previously shown that newborn trkA and trkC KO mice exhibit loss of approximately 70% and 20%, respectively, DRG neurons. In order to study the role of neurotrophin signaling through Trk receptors, we have analyzed the developmental course of primary sensory DRG neurons in knockout mice lacking either TrkA or TrkC receptors. By E11.5, 48 hours after the first DRG neurons are born, trkA expression is already detectable by in situ hybridization in newly generated DRG neurons. At this time, we did not observe differences between trkA mutant and wild-type mice. However, at E13.5 fewer neurons appear to express trkA transcripts in the trkA KO mice when compared to their wild-type littermates. Furthermore, 10% of DRG cells in the trkA KO animals exhibited fragmented DNA versus 1.2% in control mice, suggesting that TrkA-defective neurons are undergoing active cell death at this time. In agreement with these observations, skin innervation is reduced in the TrkA mutant E13.5 embryos when compared with wild-type mice. By E17.5, a complete depletion of fibers in lamina I and II of the spinal cord was observed, whereas other classes of afferent fibers were present. At this time, 80% of DRG neurons have already disappeared. These observations indicate that DRG neurons are born in the trkA KO mice, but they die shortly after the onset of the trkA gene expression. Therefore, suggesting a critical role for the TrkA receptors in neuronal survival. To determine the generality of these findings, we have studied the early development of DRG neurons in trkC mutant mice. In these mice, we observed an important reduction in the expression of trkC transcripts as early as E11.5. Furthermore, we found 3.2% of pycnotic nuclei in the trkC KO mice versus 1.3% in the wild type littermates, suggesting that increased neuronal death is occurring even at this early age. In addition to increased neuronal cell death, the trkC KO animals display several deficiencies in axonal growth when compared with their wild type littermates. For instance, at E13.5, no Ia axons have entered the spinal cord whereas at E15.5 no axons have reached the motor pools in the ventral horn. In conclusion, DRG neurons are born in trkA and trkC KO mice, however they undergo apoptotic cell death at an early developmental stage before their axons reach their central targets.