ADVANCES IN UNDERSTANDING NEURODEGENERATIVE DISORDERS

Organizers: Dennis Cunningham and Donald Price

March 28-April 4, 1992

Plenary Sessions Page

Poster Sessions

New Diagnostic Approaches

S 001 AMYLOID β-PROTEIN DEPOSITION: A SEMINAL PATHOGENETIC EVENT IN ALZHEIMER'S DISEASE, Dennis Selkoe, Harvard Medical School, Brigham and Women's Hospital, Boston, MA.

Neuronal dysfunction in AD is accompanied by progressive formation of intraneuronal and extracellular proteinaceous filaments. The intraneuronal paired helical filaments are structurally, antigenically and biochemically distinct from the extracellular amyloid fibrils and contain altered forms of tau. Amorphous, largely non-fibrillar deposits of the amyloid β-protein (AβP) precede the development of fibrillary amyloid, neuritic plaques, neurofibrillary tangles, gliosis and other cytopathological changes in AD and Down's syndrome (DS). We studied such diffuse plaques to advantage in AD cerebellum and striatum, where most of them show no neuritic component even at the end stage of AD. If local neuronal/neuritic alteration were a prerequisite for AβP deposition, one would expect some evidence of structural changes in local neurites after years of cerebellar and striatal AβP deposition. Similarly, we have detected numerous neurite-free AβP deposits in deep subcortical white matter using new antigen-enhancing techniques. We have also demonstrated occasional AβP-immunoreactive deposits in and around selected microvessels of skin, intestine, spleen and other non-neural tissues. These and other findings suggest that β-amyloidosis, like other better characterized amyloidoses, does not occur secondary to local cellular pathology but rather precedes it.

AβP is a ~40 residue fragment of βAPP, which is expressed in brain and non-neural tissues as a heterogeneous group of 110-135 kDa membrane-associated glycoproteins. We have detected a stable 22 kDa fragment of βAPP containing intact AβP selectively in brain microvessels. Further, we have recently identified in cultured primary human cells a second constitutive pathway for the processing of βAPP that involves internalization of the full-length precursor from the cell surface and targeting to lysosomes, where AβP-containing fragments are generated. Based on these and other data, we hypothesize that during aging, an alternate but normal βAPP proteolytic pathway results in the generation and release of small amounts of amyloidogenic fragments containing AβP. In DS, this alternate path is overutilized due to the higher βAPP gene dosage. In FAD, mutations or other genetic defects on ch. 21, including those in the βAPP gene itself, lead either to structurally abnormal βAPP molecules or a disregulation of βAPP synthesis that results in more processing through the alternate pathway. The result is a β-amyloid phenotype highly similar to that of DS. The progressive deposition of AβP in DS and AD initiates a slow, chronic cascade of regionally-selective cellular changes (including neuritic degeneration) that, over years, causes neuronal dysfunction and thus dementia.

Mechanisms of Neuronal Degeneration

S 002 PROTEASE NEXIN-1: A THROMBIN INHIBITOR WHICH REGULATES PROCESSES ON NEURONS AND ASTROCYTES AND IS REDUCED IN ALZHEIMER'S DISEASE. Dennis D. Cunningham, Steven L. Wagner, David Gurwitz, and Patrick Vaughan. Department of Microbiology and Molecular Genetics, University of California, Irvine, CA 92717

The protease nexins (PN-1 and PN-2) are protease inhibitors that are synthesized and secreted by several kinds of cultured cells. They are abundant in brain. The PNs form stable complexes with certain serine proteases. The complexes bind back to the cells and are rapidly internalized and degraded. This provides a mechanism for inhibiting and clearing certain serine proteases in the extracellular environment. This localized production of PNs and clearance of PN-protease complexes is well suited for the brain because the blood brain barrier prevents plasma protease inhibitors from entering the brain.

PN-1 is a 43 kDa protein that rapidly inhibits thrombin, urokinase and plasmin. PN-1 binds to the extracellular matrix and is localized there under physiological conditions. This interaction accelerates its inactivation of thrombin. The acceleration is due to heparan sulfate in the extracellular matrix. The interaction of PN-1 with the extracellular matrix blocks its ability to inhibit urokinase or plasmin. Thus, matrix-bound PN-1 is a specific and highly potent thrombin inhibitor.

PN-1 is identical to the glial-derived neurite promoting factor or glial-derived nexin described by Monard and colleagues. It stimulates neurite outgrowth in cultured neuroblastoma cells. Thrombin retracts neurites on these cells. The retraction does not occur with other proteases tested and requires only sub picomolar thrombin concentrations. The neurite outgrowth activity of PN-1 depends on its ability to inhibit thrombin; other thrombin inhibitors can also stimulate neuroblastoma neurite outgrowth. PN-1 and thrombin similarly regulate processes on cultured human fetal brain cells and cultured rat astrocytes. Thrombin is a potent mitogen for cultured astrocytes; this activity can be modulated by PN-1.

Much of the PN-1 in human brain occurs around blood vessels. In capillaries, PN-1 appears close to astrocyte foot processes. In larger blood vessels, PN-1 appears in smooth muscle cells. This suggests a protective role for PN-1 against extravasated thrombin under conditions where the blood brain barrier is altered. PN-1 is reduced about seven-fold in autopsy brain samples of individuals with Alzheimer's disease compared to age-matched control samples. The basis for this decrease is not known but appears not to be due to decreased synthesis of PN-1.

A protective role for PN-1 in brain under conditions of injury/inflammation is further suggested by studies on the regulation of PN-1 secretion by cultured glial cells. Factors which are associated with injury or inflammation such as interleukin-1, tumor necrosis factor-α, platelet-derived growth factor and transforming growth factor-β all stimulate secretion of PN-1 by glial cells. The secreted PN-1 would inhibit thrombin produced at sites of injury or inflammation. This could participate in regulation of blood coagulation and limit deleterious effects of thrombin on neurons and glial cells.

Neurol Abnormalities in AD

S 003 SEQUENCE OF MOLECULAR EVENTS LEADING TO NEUROFIBRILLARY DEGENERATION, Khalid Iqbal and Inge Grundke-Iqbal, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

The most characteristic brain lesion of Alzheimer disease (AD) is the accumulation of paired helical filaments (PHF) in the affected neurons. The PHF accumulate as neurofibrillary tangles in the cell body, neuropil threads in the dystrophic neurites in the neuropil, and as dystrophic neurites often surrounding amyloid in the neuritic(senile) plaques. Based on solubility in detergents there are two general populations of PHF, the readily soluble (PHF I) and the sparingly soluble (PHF II) types (Iqbal, et al. Acta Neuropathol. 62:167-177, 1984). Isolation of PHF in the presence of sodium dodecyl sulphate results in mostly PHF II (Iqbal, et al. 1984), in the presence of nonionic detergents in PHF I (Rubenstein, et al. Brain Res. 372:80-88, 1986), and in the absence of detergents the isolation results in both PHF I and PHF II (Iqbal, et al. Brain Res. 77:337-343, 1974). The major polypeptides of both PHF I and PHF II are the microtubule associated protein tau (Iqbal, et al. 1984; Grundke-Iqbal, et al. J. Biol. Chem. 261:6084-6089, 1986; Lee, et al, Science, 251:675-678, 1991). Tau in PHF is present in abnormally phosphorylated forms (Grundke-Iqbal, et al. Proc. Natl. Acad. sci. USA, 83:4913-4917, 1986; Iqbal, et al. ibid, 86:5646-5650, 1989, Lee, et al. 1991). In addition to the PHF, the abnormal tau is also present in unpolymerized form in the AD brain (Bancher, et al. Brain Res. 477:90-99, 1989; ibid, 539:11-18,1991; Köpke, et al. Neurosci.Abst. 422.18, 1991). Small amounts of ubiquitin (<5%) are associated with PHF II but neither with PHF I nor with the unpolymerized abnormally phosphorylated tau in AD brain (Mori, et al. Science, 235:1641-1644, 1987; Grundke-Iqbal, et al. Mol. Brain Res. 4:43-52, 1988; Lee, et al, 1991; Köpke, et al. 1991). Furthermore, the pretangle neurons are immunolabeled for abnormallyphosphorylated tau and not for ubiquitin (Bancher, et al. 1989, 1991). The level of tau in neocortex in AD brain is approximately 6-fold as high as in aged control brain, but this increase is in the form of the abnormally phosphorylated protein (Khatoon, et al. Neurosci. Abst. 422.2, 1991). The microtubule associated proteins from AD brain do not promote the assembly of microtubules in vitro (Iqbal, et al. Lancet, 2:421-426, 1986; Nieto, et al. Neurosci. 37:163-170, 1990), whereas the in vitro dephosphorylated PHF polypeptides stimulate the assembly of microtubules (Iqbal, et al. J. Neuropathol. Exp. Neurol. 50:316, 1991). In vitro the phosphate groups in PHF are less accessible than those of tau to alkaline phosphatase (Iqbal, et al. J. Neuropathol. Exp. Neurol. 49:270, 1990). It is suggested that a defect in the protein phosphorylation/dephosphorylation system is one of the early events in the neurofibrillary pathology in AD. A lack of functional tau produced by its phosphorylation and polymerization into PHF most probably contributes to a microtubule assembly defect and consequently compromises the axoplasmic flow and leads to neuronal degeneration. (Supported in part by NIH grants AG05892, AG0876, AG04220, NS18105 and a grant from the American Health Assistance Foundation).

Amyloidogenesis-I

S 004 DISEASE CAUSING MUTATIONS ALTER THE PROCESSING OF AMYLOID ß PROTEIN PRECURSOR

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Amyloid protein (AßP) accumulates in brains of patients with Alzheimer's disease (AD), Down syndrome, hereditary cerebral hemorrhage with amyloidosis - Dutch type (HCHWA-D) and to a lesser degree in brains of old individuals. AßP is a small fragment of a transmembrane glycoprotein, termed amyloid ß protein precursor (APP), which normally undergoes proteolytic cleavage within AßP region followed by secretion of a large aminoterminal part. The C-terminal half of AßP region is located within the membrane and is not accessible for proteolytic cleavage. We have expressed recombinant APPs with mutations found in AD and HCHWA-D patients and analyzed secreted and intracellular forms of APP. We found that APP can be secreted without the proteolytic cleavage of its C-terminal part. The mutations affected both secretion and degradation of APP. The amount of secreted APP with intact C-terminus appeared to increase in media of cells expressing mutated APP. In addition, mutated APP apparently underwent alternative degradation resulting in formation of intermediate low molecular C-terminal fragments with intact AßP region. These results suggest that extracellular AßP might be formed from secreted APP and alternative degradation might lead to intracellular formation of AßP. The critical step that determines the fate of APP proteins might occur during translocation of newly synthesized APP molecules across the endoplasmic reticulum membrane. APP molecules that are retained in the membrane undergo proteolytic cleavage resulting in the formation of two fragments the secreted N-terminal and the retained C-terminal. Normally only a small number of intact APP molecules would cross through the membrane. These APP molecules would have AßP region intact and both N- and C-terminal ends accessible to further proteolysis resulting in the formation of AßP. Some of these molecules may undergo secretion with subsequent degradation in the extracellular space resulting in the formation of AßP. Others may undergo intracellular degradation with the same result. The number of such molecules is small, therefore, it takes years for amyloid to reach the levels found in aged individuals. The overexpression of APP in AD and Down syndrome would increase the number of intact APP molecules that cross through the membrane resulting in earlier accumulation of amyloid. Mutations found in patients with HCHWA-D or AD might interfere with the retention of APP in the membrane resulting in the formation of AßP.

S 005 PROCESSING OF AMYLOID PRECURSOR PROTEINS *IN VITRO* AND *IN VIVO*.

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Alzheimer's disease (AD), the most prevalent mental affliction of the elderly population, is characterized by progressive loss of memory as well as other cognitive dysfunctions. The classical neuropathological hallmarks of AD are the presence of neurofibrillary tangles in neurons of the amygdala, hippocampus, and neocortex and extracellular deposits of ß amyloid in cerebral parenchyma and around blood vessels. The principal component of plaques is a *ca.* 4-kD peptide, Aß, derived from larger amyloid precursor proteins (APP). APP, encoded by alternatively spliced mRNAs, are integral membrane glycoproteins of 695, 714, 751, and 770 amino acids. The Aß peptide encompasses 28 residues of the extracellular domain and 14-15 residues of the transmembrane helix of APP. In cultured mammalian cells, holo-APPs mature through the constitutive secretory pathway, and appear at the cell surface and as C-terminally truncated APP molecules in the conditioned medium. Similar C-terminally truncated APP molecules are also observed in the cerebrospinal fluid. Using molecular biological approaches, we demonstrated that C-terminal truncation of APP occurs within the Aß region. Biochemical analysis of termini generated following cleavage confirmed that APP is cleaved between the 12th and 13th residues N-terminal to the transmembrane region. Together, these studies indicated that Aß can only be generated as an intact fragment by alternative processing pathways of APP. Recent studies have revealed that APP is only a substrate for cleavage when bound to the external side of the plasma membrane. Remarkably, efficient cleavage of precursors harboring amino acid substitutions at the normal cleavage site suggests that the protease has multiple specificities. The primary determinant of cleavage appears to be the distance from the membrane (12-13 amino acids), which is strongly dependent on local secondary structure. Our *in vivo* studies have focused on the fate of neuronally synthesized APP. Using ligation paradigms, we demonstrated that APP is axonally transported by the fast anterograde component in rat sciatic nerve. Our present studies demonstrate that in the peripheral nervous system, holo-APP-695 is the principal APP form being transported. We have examined the rat perforant pathway to assess the transport and processing of APP in the central nervous system. These studies revealed that holo-APP-695, synthesized in the entorhinal cortex, is proteolytically cleaved, in the dentate gyrus. Further studies regarding the kinetics of this process will be presented. In parallel, we have investigated the subcellular localization of APP in rat cortical membranes. Our studies demonstrate copurification of APP with synaptophysin-containing vesicles, suggesting a role for neuronal APP in synaptic vesicle metabolism. Furthermore, a large fraction of APP also copurifies with transferrin receptor-enriched membranes, indicating an endosome-related pathway for APP recycling. In this vein, we have identified sequences in the APP cytoplasmic tail that are necessary for rapid endocytosis in cultured cells. Studies examining the processing of APP in endosomal-lysosomal systems will also be presented.

S 006 PROTEASE NEXIN-2/AMYLOID β-PROTEIN PRECURSOR: PROCESSING AND PHYSIOLOGIC FUNCTIONS,

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The β/A4 protein is a 39-42 amino acid peptide that is deposited in senile plaques and in cerebral vessel walls in individuals with Alzheimer's disease (AD), Down's syndrome, and hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D). Since the β/A4 protein is derived from abnormal proteolytic processing of its parent protein, the amyloid β/A4-protein precursor (APP), we investigated if levels of the secreted forms of APP are altered in the cerebrospinal fluid of AD patients. Quantitative immunoblotting and enzyme-linked immunosorbent assays revealed marked decreases in the cerebrospinal fluid levels of APP in probable AD patients compared to age-matched patients with non-Alzheimer's type dementia or nondemented, healthy individuals. Individuals with HCHWA-D have a point mutation in their APP gene which results in a glutamine for glutamic acid substitution at position 22 in the β/A4 protein domain of APP. This mutation has been postulated to play a role in the abnormal proteolysis of APP leading to the formation and deposition of the β/A4 protein in this disease. Similar to our findings with the probable AD patients, individuals with the HCHWA-D mutation and clinically manifesting the disease also exhibited decreased cerebrospinal fluid levels of soluble APP. These findings further establish a pathogenic link between AD and HCHWA-D and suggest a common biochemical marker for these two diseases. In addition, individuals with HCHWA-Icelandic type have a point mutation in their gene encoding cystatin C, a cystiene protease inhibitor, which leads to altered proteolysis of the protein, deposition of an amyloid fragment in the cerebral vessel walls, and to decreased cerebrospinal fluid levels of cystatin C. Together, these studies indicate that decreased cerebrospinal fluid levels of amyloid precursor proteins represent a common feature of diseases that involve cerebrovascular deposition of amyloid. Our studies have also centered on elucidating the physiological function of protease nexin-2 (PN-2) which is the secreted form of APP that contains the Kunitz protease inhibitor domain. PN-2 is a potent inhibitor of several trypsin-like serine proteases including intrinsic blood coagulation factor XIa. Recent reports have indicated the importance of factor XIa as a key protease involved with sustaining the blood coagulation process. Previous studies by us showed that PN-2 is an abundant platelet α granule protein and is secreted upon platelet activation. Thus, platelets provide an effective systemic mechanism to deliver large amounts of PN-2 to sites of vascular damage. Together, these properties suggest that secreted platelet PN-2 plays a likely role in modulating blood coagulation, through factor XIa inhibition, during the wound repair process at sites of vascular injury. Other recent studies have suggested that PN-2, in concert with the potent antithrombin PN-1, may also serve as a local anticoagulant molecule in the brain. This notion is supported by the findings that 1) the cerebrovasculature lacks thrombomodulin, an important anticoagulant molecule that is present throughout the systemic vasculature 2) both PN-2 and PN-1 are far more abundant in brain than in other tissues, and 3) both PN-2 and PN-1 exhibit a perivascular localization in the brain.

Amyloidogenesis-II

S 007 ACCELERATED INSTRUCTIVE FIBRILLOGENESIS IN THE DUTCH VARIANT OF ALZHEIMER'S DISEASE, Blas Frangione, Thomas Wisniewski, Jorge Ghiso, New York University Medical Center, New York, NY 10016.

The deposition of amyloid ß (Aß) as fibrils in brain is the central event in a number of diseases, including Alzheimer's Disease (AD) and Hereditary Cerebral Hemorrhage with Amyloidosis - Dutch type (HCHWA-D). Vascular Aß is a 39-residues polymerizing peptide derived by inhibition of or defective normal proteolytic cleavage of a larger membrane glycoprotein, amyloid precursor protein (APP). A mutation found in the APP gene of patients with HCHWA-D at codon 618 (APP_{695}) causes a glutamine for glutamic acid substitution at position 22 of Aß. Although the influence of this mutation in fibrillogenesis is not known, it is clear that affected patients have accelerated cerebrovascular amyloid deposition, with disease symptoms appearing early in life. Significantly, amyloid from Dutch patients' vessel contains both the mutated and non-mutated Aß. Study using synthetic peptides homologous to Aß shows the mutation accelerates fibrillogenesis. Moreover, studies combining synthetic peptides with and without the mutation suggest fibrillogenesis is accelerated in both. These findings provide a model for accelerated amyloid formation in the Dutch variant of APP and suggest that the variant instructs the normal protein to adopt the abnormal configuration of the amyloid fibril.

Animal Models for AD and SE

S 008 AGED NONHUMAN PRIMATES: AN ANIMAL MODEL OF AGE-ASSOCIATED NEURODEGENERATIVE DISEASE. Donald L. Price, Lee J. Martin, Sangram S. Sisodia, Lary C. Walker, Vassilis E. Koliatsos, and Linda C. Cork, Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, Maryland

Rhesus monkeys, which have a potential lifespan of >30 years (i.e., human equivalent of >90 years), exhibit declines in performance on cognitive and memory tasks that parallel those manifest by aged humans. These impairments appear at the monkey equivalent of late-middle life. As occurs in elderly humans, significant differences exist in levels of performance between individual old animals. Patterns of impaired performance of some tasks (with sparing of other behaviors) are consistent with the idea that certain brain regions and neural circuits are differentially affected by age-associated pathological processes. Old animals show many of the cellular abnormalities that occur in the brains of aged humans and, to a greater extent, individuals with Alzheimer's disease (AD). Many neurons show degenerative changes, including the formation of abnormal axons and terminals (neurites). At least one old animal developed tangle-like inclusions in cortical neurons. Aβ-amyloid, a 4-kD peptide derived from the amyloid precursor protein (APP), is deposited in senile plaques and around blood vessels. APP-enriched neurites are often in proximity to Aβ-positive deposits, suggesting that these two abnormalities are related. Our recent studies have shown that APP is rapidly transported in axons and that some APP reaches nerve terminals, where it is presumed to play a role in synaptic interactions. We postulate that diseased terminals process APP aberrantly, leading to abnormal accumulation of APP within membranes of neurites; failure of the normal cleavage of APP within the Aβ domain is followed by abnormal cleavages that generate amyloidogenic fragments that self-assemble into fibrils in brain parenchyma. In some older animals, decrements occur in markers of specific neurotransmitter circuits, including the basal forebrain cholinergic system. Alterations in these cholinergic neurons contribute to the memory deficits that occur in older animals. Because axotomy-induced retrograde degeneration of these neurons can be ameliorated by the administration of nerve growth factor (NGF), it is critical to determine whether the administration of NGF improves performance of these aged animals on memory tasks.

S 009 MOLECULAR GENETICS AND TRANSGENIC MODEL OF GERSTMANN-STRÄUSSLER-SCHEINKER DISEASE, K.K. Hsiao, D. Groth, M. Scott, H. Serban, S.L. Yang, D. Rapp, D. Foster, M. Torchia, S. DeArmond, and S.B. Prusiner. University of California, San Francisco 94143.

Gerstmann-Sträussler-Scheinker disease (GSS) is a rare, dominantly inherited neurodegenerative disease that can sometimes be transmitted to experimental animals through intracerebral inoculation of brain homogenates from patients. It was recently shown that a leucine substitution at prion protein (PrP) codon 102 causes GSS in some families, since the mutation is genetically linked to the disease and causes the spontaneous appearance of a fatal spongiform neurodegeneration similar to murine scrapie in transgenic (Tg) GSS mice. Although a protease-resistant isoform of PrP is present in rodents with experimental scrapie, symptomatic TgGSS mice which express a mutant PrP with leu_{101} (homologous to leu_{102} in humans with GSS) express little or no protease-resistant PrP on Western blots. We now have evidence that the disease produced in TgGSS mice can be transmitted through inoculation of brain extracts derived from affected TgGSS mice. Intracerebral inoculation of a 5% brain homogenate derived from the founder TgGSS mouse, which developed ataxia, lethargy, and rigidity at 234 days of age, produced neurologic dysfunction in 8/11 Tg(*Prn-p*^{b}) mice, which express high levels of a murine variant PrP gene, at 259±8 days and in 2/4 Tg(SHaPrP) mice, which express high levels of Syrian hamster PrP, at 397±0 days. Inoculation of a 10% brain homogenate derived from an offspring of the founder TgGSS mouse, ill at 170 days of age, produced neurologic symptoms of experimental scrapie in 7/10 Syrian golden hamsters at 221±26 days and in 4/12 Tg(SHaPrP) mice at 196±8 days. Swiss CD-1 mice inoculated with either homogenate and uninoculated Tg(*Prn-p*^{b}) and Tg(SHaPrP) mice and hamsters remain free of neurologic disease at >390 days of age. Neuropathologic studies and analyses for protease-resistant PrP revealed prominent spongiform degeneration in a novel topographic distribution in Syrian hamsters and Tg(SHaPrP) mice inoculated with brain homogenate from the offspring and the presence of protease-resistant PrP in Syrian hamsters; more subtle spongiform changes and no detectable protease-resistant PrP were observed in Tg(*Prn-p*^{b}) mice inoculated with brain homogenate from the founder. Serial passage and end-point titration of brain homogenate from an affected Syrian hamster into Syrian hamsters produced neurologic symptoms at 75 days and a high titer. Brain homogenates of Syrian hamsters contain protease-resistant PrP, but no protease-resistant PrP is detectable in brain homogenates of Tg(*Prn-p*^{b}) mice inoculated with brain homogenate from the founder or Tg(SHaPrP) mice inoculated with brain homogenate from the offspring. Syrian hamsters inoculated with brain homogenates from 2 Tg(SHaPrP) mice inoculated with brain homogenate from the offspring, and Swiss CD-1 mice inoculated with brain homogenates from 2 Tg(*Prn-p*^{b}) mice inoculated with brain homogenate from the founder remain free of neurologic disease at >119 days of age. Serial passage to Tg(SHaPrP) mice of Tg(SHaPrP) mice inoculated with brain homogenate from the offspring remains negative at 63 days. While prolonged incubation times can be indicative of contamination with low levels of scrapie prions, they are also consistent with the low levels of protease-resistant PrP in the brains of affected TgGSS mice. The production of prions *de novo* in TgGSS mice argues that prions lack foreign nucleic acid. The low or absent levels of detectable protease-resistant PrP and the low apparent level of prions suggest that neuronal death in TgGSS mice may occur, at least in part, through abnormal metabolism of mutant PrP.

S 010 TRISOMY16 GRAFTS - A MOUSE MODEL FOR ALZHEIMER'S DISEASE AND DOWN SYNDROME RELATED NEUROPATHOLOGY.

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Alzheimer's disease (AD) is the commonest form of dementia. Trisomy 21 individuals (Down syndrome) are known to be at risk of developing AD in middle life[1]. This has been attributed to the presence of gene(s) on chromosome 21 which in excess lead to the neuropathological changes observed in AD. The main neuropathological features of AD are amyloid plaques and neurofibrillary tangles, which occur at highest density in the neocortex and hippocampus[2,3]. Trisomic strains of experimental mice can be derived using breeding regimes which select for specific Robertsonian translocations, and this has enabled gene dosage effects to be studied *in vivo*. Developments in cytogenetic techniques now permit mapping of single copy DNA sequences onto animal chromosomes[4]. In particular, human chromosome 21 sequences associated with the amyloid precursor protein (APP) and D21S16 (the closest marker associated with familial Alzheimer's gene) have been mapped to murine chromosome 16[5]. Consequently it is interesting to ask whether over-expression of genes on chromosome 16 in the Trisomy 16 mouse would produce similar neuropathological changes to those observed in the human disease and individuals with Trisomy 21. Until recently it has not been possible to address this issue since Trisomy 16 mice rarely survive beyond day 18 of gestation. However, techniques are well established for the transplantation of foetal neural tissues into adult recipient brain where the donor tissue readily survives and develops for the duration of the host lifespan. In a preliminary investigation, Trisomy 16 murine cortical tissues were examined histologically and immunocytochemical up to 6 months post-transplantation. At 4 months survival, trisomic tissues revealed cellular accumulations of β/A4 and tau proteins. In particular the presence of a tau epitope recognising the antibody Tau 6.423 which is specific to the configuration of tau observed in human AD strongly supported this system as a model for the neuropathology of AD. Furthermore, the absence of these β/A4 and tau accumulations in the Trisomy 16 embryo, grafted trisomic tissues up to 4 months post-transplantation and control euploidic grafts of the same age, indicates that the pathology observed within these trisomic grafts occur with ageing[6]. Further immunocytochemical assessment at greater than one year survival has revealed amyloid immunoreactive staining within a few of the control grafts. Immunocytochemical and ultrastructural analysis supporting this neural transplantation model will be presented.

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Therapeutic Strategies-I

S 011 GRAFTING GENETICALLY MODIFIED CELLS TO THE BRAIN,

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To assess the feasibility of using primary skin fibroblasts as a donor population for genetic modification and subsequent intracerebral grafting, we examined the structural and neurochemical characteristics of grafts of isogeneic primary fibroblasts over a period of six months. Following the implantation in adult rats from the same inbred strain, isologous grafts are stained immunohistochemically for fibronectin immunostaining which persists for at least six months. Immunostaining for laminin is intense within the grafts from one to eight weeks, but decreases by six months. Astrocytes respond dramatically to the implantation of primary fibroblasts although the intensity of immunostaining for glial fibrillary acidic protein diminishes between eight weeks and six months. The astrocytic border between the grafts and striatal neuropil remains intensely immunostained. Capillaries within the grafts stain immunohistochemically for glucose transporter as early as three weeks after implantation. At the ultrastructural level, grafts possess numerous fibroblasts and have an extracellular matrix filled with collagen. Reactive astrocytic processes filled with intermediate filaments are found throughout the grafts. Hypertrophied astrocytes and their processes also appear to form a continuous border between the grafts and the striatal neuropil. Grafts of primary fibroblasts also possess an extensive vasculature that is composed of capillaries with nonfenestrated endothelial cells; the occurrence of reactive astrocytic processes closely associated with or enveloping capillaries is variable. These results provide direct morphological and neurochemical evidence for the long-term survival of isologous fibroblasts after intracerebral implantation. We have transduced primary isologous fibroblasts with several different transgenes to assess the effectiveness of these genetically modified cells to delivery new genetic material to the brain, including Nerve Growth Factor (NGF), Tyrosine Hydroxylase, GABA Decarboxylase, and Choline Acetyltransferase. We will report on intracerebral grafts of primary skin fibroblasts genetically engineered to expressing several of these transgenes, including NGF which had been embedded within a collagen matrix prior to grafting. This later experiment was designed to assess the regenerative capacity of cholinergic neurons of the adult rat medial septum. The results reveal the following: first, NGF-producing grafts sustain a significant number of NGF receptor-immunoreactive septal neurons following axotomy. Second, NGF promotes the regeneration of septal axons, such that NGF-producing grafts possess large numbers of unmyelinated axons which use many a variety of substrates for growth. Grafts of control fibroblasts possess the same cellular and matrix substrates but contain only a very small population of axons. Advantages and disadvantages of these grafted genetically modified primary fibroblasts will also be discussed.

S 012 NEUROTROPHIC STRATEGIES IN NEURODEGENERATIVE DISEASE,

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Several groups of protein growth factors, called neurotrophic factors, regulate *developmental survival and growth of neurons and are involved in the regulation of* neuronal survival, plasticity and regeneration in the adult nervous system. The discovery of neurotrophic factors offers an avenue to develop effective therapy for disorders associated with degeneration and loss of neurons. They have the potential to lead to the establishment of a new, structurally oriented neuropharmacology different from the established neuropharmacology which is based on drugs influencing mechanisms related to neuronal impulse flow and transmission at the synapse.

Cell culture studies with dissociated primary cultures from embryonic rat brain revealed that brain-derived neurotrophic factor (BDNF) promotes the developmental differentiation of both, basal forebrain cholinergic and mesencephalic dopaminergic neurons. These studies suggested that BDNF may be able to protect cholinergic and dopaminergic neurons from degenerative changes induced by axotomy in the adult brain, similar to the known protective action of nerve growth factor (NGF) on cholinergic neurons. We found that intraventricular administration of recombinant human BDNF to adult rats with transections of the fimbria significantly reduces axotomy-induced degenerative changes of the cholinergic cells in the basal forebrain. No similar effect was seen on the dopaminergic neurons of the ventral mesencephalon after transection of their axons ascending in the medial forebrain bundle. The protective effect of the intracerebroventricular injections of BDNF on cholinergic neurons of BDNF was evident only for part of the population of cholinergic neurons affected by the lesion, whereas the entire population was protected by NGF treatment.

The pharmacological administration of neurotrophins involves several problems, in particular, its administration to the brain will require surgical implantation of an intracranial delivery device. Such problems could be overcome by the availability of low-molecular compounds affecting neurotrophic functions, whose biophysical properties can be chemically modified more easily than those of proteins. We obtained evidence suggesting that it will be feasible to develop compounds selectively modifying the actions of individual members of the family of neurotrophin proteins. K-252b, a protein kinase inhibitor earlier shown to inhibit nerve growth factor actions on cholinergic neurons of the basal forebrain, prevented trophic actions of other neurotrophins, BDNF and neurotrophin-3 (NT-3), on central cholinergic and dopaminergic neurons, peripheral sensory neurons and PC12 cells, when used at μM concentrations. Comparable actions of non-neurotrophin growth factors were not affected. At nM concentrations, K-252b selectively enhanced the trophic action of neurotrophin-3 on central cholinergic neurons, peripheral sensory neurons, and PC12 cells. In PC12 cells, K-252b potentiated the neurotrophin-3 induced tyrosine phosphorylation of *trk*, a protein kinase responsible for transmitting neurotrophin signals. A direct action of K-252b on *trk* and *trk*B was demonstrated using partially purified recombinant proteins in a cell-free assay. These findings indicate that K-252b generally and selectively potentiates the neurotrophic action of NT-3 and that it directly acts on *trk*-type neurotrophin receptors.

S 013 NEUROTROPHIC EFFECTS ON MODEL SYSTEMS OF THE BRAIN AND SPINAL CORD. Vassilis E. Koliatsos[1], Donald L. Price[1], Richard E. Clatterbuck[1], George Yancopoulos[2], David S. Olton[3], and John W. Winslow[4], [1]Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD, [3]Department of Psychology, The Johns Hopkins University, Baltimore, MD, [2]Regeneron Pharmaceuticals, Tarrytown, NY, and [4]Genentech, Inc, South San Francisco, CA

Neurotrophic factors affect selected populations of neurons by several mechanisms, including retrograde transport from target cells in the central nervous system and periphery (neurotrophins: nerve growth factor [NGF], brain-derived neurotrophic factor [BDNF], neurotrophin-3 [NT-3]) and release from lesioned neurons (ciliary neurotrophic factor [CNTF]). We have investigated the effects of recombinant neurotrophins and CNTF on three populations of neurons, chosen on the basis of their relevance to human neurodegenerative disease. The first population includes cholinergic and GABAergic neurons of the basal nucleus complex (BNC). These cells play significant roles in learning and memory, and they degenerate early and consistently in dementing disorders, such as Alzheimer's disease (AD). Cholinergic neurons of the BNC express the $p75^{NGFR}$ and $p140^{trk}$ receptors and respond to NGF. Normal cholinergic neurons in the adult BNC increase the expression of ChAT and $p75^{NGFR}$ in the presence of NGF. Following axotomy, NGF protects cholinergic BNC neurons by maintaining normal levels of ChAT activity and immunoreactivity and $p75^{NGFR}$ mRNA; the effects of NGF on these lesioned neurons are dose dependent and saturate at the level of 2 µg/day intraventricularly. In aged behaviorally characterized Fischer-344 rats, intraventricular delivery of NGF reverses abnormalities in behaviors dependent on forebrain cholinergic transmission (i.e. spatial memory) but does not affect sensorimotor skills. These effects are associated with increases in ChAT activity in hippocampus and neocortex and increase in size of cholinergic BNC neurons. BDNF, but not NT-3, partially protects the phenotype of these cells following axotomy. However, BDNF appears to reduce ChAT and $p75^{NGFR}$ expression in intact cholinergic neurons, an effect consistent with the concept of an NGF antagonistic role at the receptor level. A second system of interest is neurons of the anterior thalamic complex, which project via the cingulum to retrosplenial cortex as part of the Papez circuit. These neurons degenerate in AD and die following axonal transection; the latter event is prevented by peripheral nerve grafts implanted in the axotomized thalamus. CNTF, a factor enriched in these nerve tissues, reproduces the protective function of the nerve grafts and may alter the glial response to the lesion. NGF, BDNF, or NT-3 does not have any effect on anterior thalamic neurons. Finally, we have investigated the potential of neurotrophins and CNTF to act on spinal motor neurons. These cells can be differentiated in two groups with distinct trophic profiles: motor neurons projecting to general somatic muscles (i.e. gastrocnemius); and motor neurons innervating the sexually dimorphic muscles of the perineum. The former express $p75^{NGFR}$ during development and following injury, and their target muscles are enriched in CNTF. The latter express $p75^{NGFR}$ throughout their life span, but their target muscles do not express CNTF and are enriched in NT-3. Sexually dimorphic spinal motor neurons represent the animal homologue of Onuf's nucleus, a population of nerve cells selectively spared in several degenerative diseases of the spinal cord, including amyotrophic lateral sclerosis. The distinct trophic features of these neurons and their interactions with androgen steroids may provide clues for the enhanced protection of these cells from various insults.

Therapeutic Strategies-II

S 014 PROTEASES AS TARGETS FOR DRUG DESIGN IN ALZHEIMER'S DISEASE, Carmela R. Abraham, Bronwyn L. Razzaboni, Gregory Papastoitsis, Kazutomi Kanemaru, Eliot Picard and Barbara Meckelein, Boston University School of Medicine, Boston, MA 02118.

Our laboratory studies the proteolytic processing of the amyloid precursor protein (APP) in the brains of humans and non human primates. There is increasing evidence that an aberrant or alternative degradation pathway of the APP causes the accumulation of the amyloid ß protein (AP) in the brains in Alzheimer's disease (AD), Down's syndrome, hereditary cerebral hemorrhage with amyloidosis of Dutch origin, and to a less extent in normal aged humans, monkeys, dogs and bears. The recently discovered mutations in APP occuring in familial AD emphasize the important role of APP in the disease process. These APP mutations also appear to affect its normal degradation. Furthermore, AP seems to be neurotoxic to neurons, both in vitro and in vivo. Thus, arresting the formation of AP may be beneficial and inhibitors that may inactivate proteases responsible for AP generation may become potential therapeutic targets for AD. Apparently, APP can be processed in a secretory pathway in which the AP region is cleaved and thus amyloidogenesis prevented. An alternative pathway must occur to generate the N and C-termini of AP. Thus far we focused our efforts on proteases cleaving at the N-terminus of AP. For initial screening of such brain proteases, we used an iodinated synthetic peptide flanking the N-terminus of AP. Following several fractionation steps of human or perfused, freshly frozen monkey brain we have obtained two major proteolytic activities. From human and monkey brain we have purified a calcium-activated serine protease very similar, but not identical, to cathepsin G in its substrate specificity and inhibitory profile. In addition to cleaving the synthetic substrate, the serine protease can also degrade purified human APP (a gift from R. Siman, Cephalon, Inc.). This 28kDa protease can be labeled on western blots with antibodies to cathepsin G. Using double-labeling, cathepsin G antibodies stain GFAP-positive cells in monkey brain mildly fixed with 70% ethanol/0.15M NaCl suggesting that astrocytes contain a cathepsin G-like protease. We then screened a fetal human brain expression library (a gift from R. Neve) with antibodies to cathepsin G and found three positive clones. Upon cDNA sequencing, all three clones were found to contain an identical 5' region but differ in the 3' region, and share a sequence motif highly conserved in serine proteases. In addition, a second protease has been purified from AD brain. This protease cleaves the synthetic peptide between methionine and aspartic acid, aspartic acid being the N-terminus of AP, and is able to degrade purified APP. Based on its inhibitory profile, the second protease is a metal-dependent cysteine protease. We hypothesize that our proteases may be involved in the alternative processing of APP and may be able to generate the N-terminus of AP. The serine protease, which is inhibited by α1-antichymotrypsin, may be secreted by astrocytes and may exert its activity extracellularly to convert preamyloid to amyloid. The cysteine protease, possibly a lysosomal protease, may attack the APP in normal lysosomes or in the lysosomes of dying neurons and neurites seen around senile plaques. The relevance of these two proteases to amyloid formation is under investigation.

Clinical Phenotypes, Genetics and Amyloidogenesis

S 100 EVIDENCE FOR SYNTHESIS OF THE SCRAPIE PRION PROTEIN IN ENDOSOMES, David R. Borchelt,[1] Albert Taraboulos,[1] and Stanley B. Prusiner[1,2], Departments of [1]Neurology and [2]Biochemistry and Biophysics, University of California, San Francisco, CA 94143

Infectious scrapie prions are composed largely, if not entirely, of an abnormal isoform of the prion protein (PrP) which is designated PrP^{Sc}. A chromosomal gene encodes both the cellular prion protein (PrP^{C}) as well as PrP^{Sc}. Pulse chase experiments with scrapie infected cultured cells indicate that PrP^{Sc} is formed by a post-translational process. PrP is translated in the endoplasmic reticulum, modified as it passes through the Golgi and is transported to the cell surface. Release of PrP from the cell surface by phosphatidylinositol-specific phospholipase C or hydrolysis with dispase prevented PrP^{Sc} synthesis. At 18°C, the synthesis of PrP^{Sc} was inhibited under conditions that other investigators have reported block of the endosomal pathway. Our results suggest that PrP^{Sc} synthesis occurs during endocytosis of PrP from the cell surface. Whether PrP^{C} or a subpopulation of PrP molecules are destined to become PrP^{Sc} remains to be established. Identifying the subcellular compartment(s) of PrP^{Sc} synthesis should be of considerable importance in defining the molecular changes that distinguish PrP^{Sc} from PrP^{C}.

S 101 HUMAN SERUM STIMULATES ALZHEIMER MARKERS IN CULTURED HIPPOCAMPAL NEURONS, Gregory J. Brewer* and J. Wesson Ashford+, *Department of Medical Microbiology and Immunology, +Department of Psychiatry, Southern Illinois University School of Medicine, Springfield, IL 62704-9230

The mechanism for promoting the distinct types of lesions in the Alzheimer disease (AD) brain and other changes outside the brain is unknown. We examined unprotected neurons in culture to determine if exposure to serum would affect markers for Alzheimer brain lesions. Rat hippocampal neurons were first grown for 4 days in a new serum-free culture medium, then exposed for 24 hr. to human sera. Sera from 12 AD patients or their spouses or, to a lesser degree, young adults each increased three molecular markers characteristic of Alzheimer senile plaques and neurofibrillary tangles: Alz-50, β-amyloid(β/A4) and MAP2, each with distinct cytologic distributions. By quantitative immunofluorescence, neuronal exposure to the elderly human sera produced 1.8 to 2.5 fold increases in mean fluorescent area/cell for each of these three markers relative to no serum exposure. As controls, an unrelated neuronal marker, enolase, was unaffected, and fetal bovine serum did not stimulate immunoreactivity. Neuron viability and somal area were unaffected. This stimulation of AD markers by human serum suggests a possible role for access or detoxification of serum factors in the genesis of both neuronal plaques and tangles.

S 102 NONDENATURING PURIFICATION OF THE NORMAL ISOFORM OF PRION PROTEIN, B. Caughey and G. Raymond, Laboratory of Persistent Viral Diseases, NIAID, NIH, Rocky Mountain Laboratories, Hamilton, MT 59840.

Our recent studies have provided evidence that the scrapie-associated conversion of prion protein (PrP) to the protease-resistant state is a posttranslational modification that occurs after PrP reaches the cell surface (Caughey and Raymond, 1991, *J. Biol. Chem.* 266:18217) and before it is translocated to lysosomes (Caughey et al., 1991, *J. Virol.* 65:6597). Since there is no known scrapie-specific covalent modification of PrP, we are investigating the possibility that this conversion of PrP involves a conformational change. Initial IR spectroscopic studies have analyzed the secondary structure of the protease-resistant PrP amyloid (Caughey et al., 1991, *Biochemistry* 30:7672). In order to compare the conformation of the amyloid form of PrP to that of the normal isoform by IR, we needed to develop a method of purifying the normal PrP under nondenaturing conditions. Thus, we have devised a immunoaffinity purification method which involved the binding of normal PrP to immobilized antibody raised against a synthetic peptide fragment of the PrP molecule. The PrP is then eluted from the matrix under nondenaturing conditions by the application of high concentrations of the synthetic PrP peptide. The resulting eluate contains PrP that is nearly pure according to SDS-PAGE. Attempts to scale up this procedure to generate sufficient quantities of normal PrP for infrared analysis are in progress.

S 103 A MUTANT AMYLOID PRECURSOR PROTEIN DEFECTIVE IN SECRETION.

John T. Durkin, Tatjana Loh, Mary J. Savage, Richard W. Scott and Robert Siman. Cephalon, Inc., West Chester, PA 19380

The amyloid plaques deposited in the brains of Alzheimer's Disease patients are largely composed of the beta/A4 peptide. Normal processing and secretion of the precursor to this peptide, the Amyloid Precursor Protein (APP), involves cleavage within the beta/A4 domain. Amyloid deposition and subsequent disease therefore require processing of APP by other pathways. We are modelling aberrant processing of APP by expressing APP mutants in cultured cell lines. The mutants examined include those that lead to amyloid deposition in human disease, and non-conservative substitutions at or near the normal cleavage site. Full-length cDNA for the 751 amino acid form of APP was cloned from a human temporal cortex library. Specific mutations were introduced by PCR mutagenesis, and the clones inserted into a CMV expression vector. These DNAs were transfected into 293 cells by $CaPO_4$ co-precipitation. Thirty-six hours after transfection, conditioned media and cell lysates were collected, separated by SDS-PAGE, blotted onto nitrocellulose and probed with antibodies against the amino or carboxyl terminal domains of APP. Newly synthesized APP, fully-glycosylated APP and secreted APP were quantitated by laser densitometry. A non-conservative mutation at the cleavage site, K612E (numbered according to APP695), changes the charge at the cleavage site but is not defective in secretion: the ratio (secreted APP in medium)/(fully-glycosylated APP in lysate) is not significantly less for the mutant than for the wild type. When this mutation is combined with a second non-conservative mutation *downstream from the normal cleavage site, the double mutant is* defective in secretion. The fate of mutant APP as it builds up in the cells, and the fragments produced as it is cleared, will be discussed.

S 104 HEAD TRAUMA AS A TRIGGER FOR ß/A4 AMYLOID PROTEIN DEPOSITION IN THE BRAIN, Stephen M. Gentleman, Alison Lynch, David I. Graham and Gareth W. Roberts Department of Anatomy and Cell Biology, St Mary's Medical School, Imperial College Institute of Science Technology and Medicine, London, W2 1PG, UK.

We have previously demonstrated that both single and repetitive head trauma are associated with the presence of diffuse ß/A4 amyloid protein plaques in the brains of long term survivors. On the basis of this data we have proposed that head trauma can trigger Alzheimer's disease. Many recent epidemiological studies support this hypothesis.

To extend our work on the aetiology of AD we have investigated 80 patients (age range 17 mnths-80 yrs) who suffered a fatal head injury and had survival times ranging from < 24 hrs-9 mnths. Sections were immunostained with an antibody to the ß/A4 protein, which is the principal component of the senile plaques of AD. We found significant deposits of the protein in the cortical ribbon of 20% of the cases studied. The youngest positively stained case was a 24 year old woman who survived for 3 days after her injury. Such deposits were not seen in age and sex matched controls. This deposition of ß/A4 protein in the brains of short lived head injury patients has never been previously described. We conclude that induction of the amyloid precursor protein in the brain may be a normal response to neuronal stress.

Our study demonstrates that severe head injury can trigger widespread but focal deposition of ß/A4 protein within days. This data supports our proposal that head trauma may be an important aetiological factor in AD.

This work was supported by an MRC project grant.

S 105 IDENTIFICATION AND CHARACTERISATION OF MUTATIONS IN THE AMYLOID PRECURSOR PROTEIN GENE WHICH CO-SEGREGATE WITH FAMILIAL ALZHEIMER'S DISEASE, Alison M.Goate, Michael J. Mullan, Marie-Christine Chartier-Harlin, Jeremy Brown, Fiona Crawford, Liana Fidani, Khalid Hamandi, David Hughes, Karen Rooke, Penelope Roques, Martin Rossor, Chris Talbot & John Hardy, Alzheimer's disease research group, Depts. of Biochemistry & Molecular Genetics & Neurology, St. Mary's Hospital Medical School, Norfolk Place, London, U.K. W2 1PG.

We recently reported a point mutation in codon 717 of the 770 amino acid transcript of the amyloid precursor protein gene in two families with early onset Alzheimer's disease (Goate, A.M. et al. Nature (1991) 349: 704-706). Data will be presented on the subsequent screening of both sporadic and familial cases for the occurrence of this mutation. It has been not been observed in over 50 familial cases of Caucasian origin tested but has been detected in several Japanese families (Naruse, et al. The Lancet (1991) 337: 978-979). A preliminary comparison of the clinical features of the families carrying the APP717(ILE) mutation indicates a similarity in the age of onset of the families and in some of the prodromal features which may enable the distinction of these families from those not carrying the mutation.

Highly informative polymorphic markers close to or within the APP gene are being used to exclude the APP gene as the site of the disease causing mutation in other early onset families suitable for linkage analysis. A third family has been identified which gives a positive lod score with these markers. Affected members of this family also have a point mutation in codon 717 leading to a valine to glycine substitution. The significance of a second mutation in codon 717 leading to Alzheimer's disease will be discussed. Data on the frequency of this mutation will be presented. Other families in which APP cannot be excluded are being screened by single stranded conformation polymorphism (SSCP) analysis and PCR direct sequencing to look for new mutations.

Some families clearly exclude APP as the disease locus demonstrating genetic heterogeneity even amongst early onset families.

S 106 THE MOLECULAR DETERMINANTS OF AMYLOID DEPOSITION IN ALZHEIMER'S DISEASE: REEVALUATING THE STRUCTURE OF THE CROSS-ß FIBRIL Kurt J. Halverson, Michele Auger, Robert G. Griffin and Peter T. Lansbury, Jr.*, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

One of the major hallmarks of the Alzheimer's disease brain is the presence of extracellular amyloid plaques. These plaques, which are extremely insoluble, arise from the self-association of a 42 amino acid protein known as the amyloid-ß protein. In order to understand the forces which govern the extraordinary stability of the plaques, it is necessary to determine the structure of the ß-protein aggregate on a molecular level. The current model for the structure of the ß-protein is the cross-ß fibril, which is composed of stacked ß-pleated sheets arranged with the hydrogen-bonding direction of the sheets parallel to the axis of the fibril. The cross-ß fibril has traditionally been defined using the techniques of X-ray fiber diffraction, electron microscopy, and FT-IR, which provide little information beyond indicating the presence of ß-sheet structure. We are interested in developing techniques which will provide a higher resolution structure of the cross-ß fibril formed by the ß-protein.

For our structural model, we are using a synthetic peptide which corresponds to the C-terminal nine amino acids of the ß-protein (H_2N-LMVGGVVIA-CO_2H; ß34-42). This peptide, derived from the region of the ß-protein which we believe nucleates *in vivo* aggregation, is extremely insoluble and also satisfies the definition of the cross-ß fibril as outlined above (*Biochemistry* **1990**, *29*, 2639). By the synthetic replacement of specific ^{12}C carbonyl atoms with ^{13}C, we are able to observe individual carbonyl absorptions in this peptide using FT-IR. The results of these experiments indicate that there are structural irregularities in the central region of ß34-42 which are not consistent with the cross-ß fibril model. Using the solid-state NMR technique of rotational resonance (R^2), which measures the distance between pairs of synthetically incorporated ^{13}C atoms, we have determined that the Gly37-Gly38 amide bond is in the unusual cis conformation. Other measured distances along the backbone of this peptide are also inconsistent with the structure predicted by the cross-ß fibril model. We are currently using the information from these experiments to build a detailed structural model of the ß34-42 aggregate, which should provide an understanding of the intra- and intermolecular interactions which may be responsible for the unusual stability of amyloid plaques.

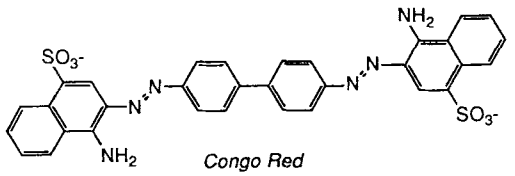
S 107 A CHICKEN PRION PROTEIN UNDERGOES TWO POST-TRANSLATIONAL CLEAVAGES. D.A. Harris. Dept. of Cell Biology & Physiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

We have recently identified chPrP, the chicken homologue of the mammalian prion protein PrP^C (Harris, et al., Proc. Natl. Acad. Sci. USA, 88:7664-7668, 1991). ChPrP is identical to mouse PrP^C at 33% of its amino acid positions, including a stretch of 24 identical residues, and it displays the same structural domains. We have used sequence-specific antibodies to detect chPrP molecules by immunoblotting and immunoprecipitation in stably transfected lines of mouse neuroblastoma (N2a) cells that express the recombinant chicken protein, as well as in subcellular fractions of chicken brain and in cerebrospinal fluid. ChPrP is anchored to the surface of transfected cells by a glycosyl-phosphatidylinositol (GPI) anchor, since it can be released from intact cells with bacterial phosphatidylinositol-specific phospholipase C (PIPLC). Two fragments of chPrP having molecular weights of 35-45kD and 11.5kD are also released into the culture medium in the absence of added enzyme. Both soluble fragments react with antibodies specific for the N-terminus (lys^{25}-gly^{35}), as well as the proline-glycine repeat region (pro^{47}-pro^{65}). The 35-45kD form cannot be metabolically labeled with ^3H-ethanolamine, suggesting that it lacks the GPI anchor structure. ChPrP is also anchored to microsomal membranes from chicken brain by a GPI linkage, and can be partially released by PIPLC. In addition, 35-45kD and 8.5-11.5kD fragments of the protein are present in a postmicrosomal fraction of brain, and in cerebrospinal fluid. We propose that the chPrP molecule undergoes at least two cleavages as part of its normal metabolism: one very near the GPI anchor attachment site, and one just N-terminal to the central hydrophobic domain. The second cleavage is within a region that is highly conserved between chPrP and mammalian PrP, and in which amino acid substitutions have been associated with variations in the development of amyloid plaques in rodent scrapie.

S 108 STUDIES OF THE INTERACTION OF CONGO RED WITH FRAGMENTS OF THE β/A4 PROTEIN

Brian F. McGuinness and Peter T. Lansbury, Jr.,* Department of Chemistry, Massachusetts Institute of Technology, Cambridge MA 02139

Alzheimer's disease (AD) is characterized by the presence of extracellular plaques or amyloid in the afflicted brain. The stain congo red (CR) selectively binds to the antiparallel β-sheet structure of amyloid. We are interested in exploiting this interaction for the long term goal of designing *in vivo* probes for the diagnosis of AD. The ultimate rational design of these probes requires the initial determination of the structure of the CR/amyloid complex in molecular detail. Toward this goal, we are screening CR analogs for their ability to bind cross-β fibrils. These CR analogs are designed to test the spatial as well as conformational constraints of the dye/amyloid interaction.



Congo Red

In addition, we are assaying a set of peptide fragments of the β/A4 protein to determine what features of this protein (aside from its β-structure) complex CR.

S 109 GENETIC LINKAGE STUDIES AT THE APP LOCUS IN EARLY AND LATE ONSET FAMILIAL ALZHEIMER'S DISEASE

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The identification of a major locus causing familial Alzheimer's disease (AD) (Goate, Harlin, Mullan et al, Nature, 1991;349:704-706) raises questions as to the extent to which other familial AD is caused by a mutation or variation in the APP gene. To this end we employed highly polymorphic repeat sequences to generate linkage data near to the APP gene in 13 early and 13 late onset familial AD families. A comparison of the lod score with simulated linkage data identified one early onset pedigree as linked to the APP gene. Subsequent sequencing revealed a second mutation at the APP locus (Harlin, Crawford, Houlden et al, Nature, 1991 in press). None of the other early (<65yrs) or late onset AD families have significant lod scores (when compared to simulated data or by conventional criteria) at the APP locus. In addition, as the mode of inheritance and penetrance in late onset AD are indeterminate, we employed the affected pedigree member method to assess the contribution of APP in the late onset families.

S 110 THE INVOLVEMENT OF NON-NEURONAL CELLS IN THE AMYLOID DEPOSITION OF ALZHEIMER'S DISEASE

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Although neurons are the cells most obviously affected in Alzheimer's disease, glia make important contributions to, and may be primarily responsible for, the amyloid deposition that is pathognomonic of the disease and likely causes the neuronal cell death. Specifically we have found that reactive astrocytes in the vicinity of the amyloid deposition of Alzheimer's disease are expressing one of the amyloid protein components—a protease inhibitor termed α_1-antichymotrypsin (ACT). ACT is a normal serum protein synthesized in the liver and overexpressed as part of the body's "acute phase response" to inflammation in the periphery. Evidently the brain also exhibits an acute phase response to neurodegeneration that involves reactive astrocytes and includes the *de novo* expression of ACT. We have found that astrocytes cultured in serum-free medium can be induced to express ACT by exposure to acute phase inducing agents, just as are hepatocytes in culture, again indicating the parallels between the peripheral and brain "acute phase responses." These inducing agents are present in increased amounts in Alzheimer's and Down syndrome brain. In addition, several chymotrypsin-like proteases have been identified in mast cells and microglia, which have the substrate specificity necessary to generate the β-protein from its precursor. Together these data indicate that the neuropathology of Alzheimer's disease may result from an imbalance in the normal neuronal-glial interaction in the brain, finally resulting in neuronal cell death.

S 111 DISTRIBUTION OF β/A4 PROTEIN AND AMYLOID PRECURSOR PROTEIN IN HCHWA-D AND ALZHEIMER'S DISEASE

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Brain amyloidosis with abundant β/A4 protein deposition in plaques and cortical and meningeal vessels (cerebral congophilic angiopathy, CAA) is found in Alzheimer's disease (AD). Several types of β/A4 plaques have been described a.o. the classical amyloid plaques surrounded by dystrophic neurites and glial cells and the "amorphous" or "diffuse", non-congophilic plaques which seem to be early stages in amyloid plaque formation. CAA and a small number of plaques has also been reported in hereditary cerebral hemorrhage with amyloidosis-Dutch type (HCHWA-D). In contrast to AD, no neuritic pathology or classical congophilic plaques are found in HCHWA-D. Therefore, these plaques can be used as a model of "amorphous" or "diffuse" plaques. Unlike most AD cases the congophilic angiopathy in HCHWA-D is very severe. It is still unknown whether β/A4 deposits in plaques and vessels have the same origin. In this study we have used frozen cortical tissue of HCHWA-D and AD patients to investigate the β/A4 amyloid protein and the amyloid precursor protein (APP) in different types of plaques and vascular amyloid. Immunohistochemical staining was conducted using against synthetic β/A4 proteins and antibodies against APP including mAbP2-1, a monoclonal antibody against purified protease nexin-2 which is the secreted form of APP. In contrast to former studies on formalin fixed, paraffin-embedded tissue we have found a large number of β/A4 positive plaques in the cortex of HCHWA-D cases, meeting the Khachaturian criteria for AD. Immunohistochemical staining on adjacent sections and doublestaining with mAbP2-1 and β/A4 antisera revealed 1) the absence of APP immunoreactivity in amorphous plaques in HCHWA-D and AD, 2) the presence of APP immunoreactivity in classical plaques and transitional forms, and 3) APP immunoreactivity in congophilic vessels in HCHWA-D but not in vascular amyloid in AD. Together these findings suggest that there is a quantitative difference in vascular amyloid formation in HCHWA-D and AD. Furthermore, the presence of APP in plaques seems to be associated with neuritic alterations. No relationship was found between plaques and congophilic vessels.

S 112 EXPRESSION OF WILD TYPE AND MUTATED HUMAN CJD AMYLOID PRECURSOR (PRION PROTEIN) IN INSECT CELLS, Jiri Safar[1], Ramaninder Bhasin[2], Larry Horn[1], Elisabeth A. Barnes[2], Dmitry. Goldgaber[2] and Clarence J. Gibbs Jr.[1], Laboratory of Central Nervous System Studies[1], NIH, Bethesda, MD 20892; Department of Psychiatry and Behavioral Science[2], SUNY, Stony Brook, NY 11794-8101.

Using the baculovirus expression vector system, we have expressed wild-type human prion protein gene (PRIP gene) and two mutations, one at codon 102 (Pro to Leu), linked to development of Gerstmann-Straussler-Scheinker's syndrome (GSS), and the other at codon 200 (Glu to Lys), associated with development of familial Creutzfeldt-Jakob disease (CJD). The recombinant proteins were detected two days post-infection and remained associated with the insect cells. The Western blot pattern of triplet bands was similar to that of normal human brain PrP^{C} with slightly lower apparent Mr of 30, 28 and 26 kDa. All recombinant proteins were partially resistant to proteolysis by proteinase K, but the pattern of immunoreactive peptides and cleavage kinetics did not correspond either to human brain PrP^{CJD} or to PrP^{C}, suggesting an alternative tertiary structure. We observed no difference in kinetics and physicochemical behavior between the wild-type recombinant PrP or those carrying mutations in codons 102 or 200. Endoglycosidase treatment reduced the triplet to one major band indicating that the bands reflects 2, 1 or 0 Asn-linked oligosaccharide chains. The susceptibility to Endo H suggests the presence of mannose-rich, simple sugar chains in contrast to the complex, highly branched chains with a terminal neuraminic acid in PrP27-30 of brain origin. The physicochemical behavior of the recombinant proteins and negative results of PIPLC cleavage indicated that human recombinant PrP proteins maintained a C-terminal hydrophobic domain.

Neuronal Degeneration

S 200 CHARACTERIZATION OF THE MAJOR NEUTRAL PROTEINASES FROM THE HIPPOCAMPUS OF ALZHEIMER AFFECTED AND CONTROL BRAIN SPECIMENS, Jon R. Backstrom, Carol A. Miller, Florence M. Hofman and Zoltan A. Tokes, Department of Biochemistry and Pathology, University of Southern California, Los Angeles, CA 90033

Non-lysosomal, neutral proteinases were investigated in postmortem hippocampal samples from ten Alzheimer affected and five control brain specimens. Substrate gel electrophoresis was used to identify and to quantitate SDS-soluble enzymes using gelatin or alpha-casein copolymerized with acrylamide. Quantitative spectrophotometry was used to assess the amount of substrate hydrolyzed in the gel under established conditions. Four regions of enzyme activity were detectable with gelatin as substrate; one migrated as a high molecular weight complex (~280,000), and the others as 130,000, 100,000, and 70,000 mol. weight enzymes. None of these enzymes showed activity against casein. The chelating agents EDTA and 1,10-phenanthroline eliminated their gelatinase activity. In the presence of Ca^{2+}, Zn^{2+} reestablished the activities of the enzymes at lower concentrations than Co^{2+} or Mn^{2+}, indicating that all of the activities were generated by metalloproteinases (MPs). The high mol. weight MP complex was detected primarily in the Alzheimer disease (AD) specimens. MP-130 was detected in 9 of the 10 AD specimens and only trace amounts were found in 1 of the 5 control specimens. MP-100 activities were about four times higher in AD specimens as compared to the controls, whereas MP-70 activities were comparable in the two groups. Activation with p-aminophenylmercuric acetate (p-APMA) resulted in a significant increase in MP-70 activities, but not MP-100 or MP-130 activities, from both AD affected and control samples. Furthermore, p-APMA treatment did not result in additional activities from either group. The activities of MP-130 and MP-100 did not correlate with the presence of mature neuritic plaques in AD specimens. Preliminary immunohistological studies indicated that MP-100 reactivity was associated predominantly with glial cells and to a limited extent with endothelial cells and neurons. These experiments establish that MPs, capable of degrading tissue matrix components and amyloid proteins, have a markedly different distribution in the hippocampus of AD affected patients which may contribute to the neuropathogenesis of the disease. (Supported by NIA R01-AG 09681).

S 201 CALPAIN ACTIVATION IN A MODEL OF STROKE, Raymond T. Bartus, Reginald Dean, Deborah Henson-Snow, Kathleen Cavanaugh, Jill Miotke, Stephen Espitia, Juan Estrtada, and David Eveleth, Cortex Pharmaceuticals, Inc., Irvine, Ca. 92718.

Stroke is the third leading cause of death in the US and a condition for which there is presently no effective treatment. Our understanding of the molecular mechanisms underlying stroke pathology has increased enourmously in recent years and it is now clear that stroke is much more complex than simple anoxia. Elevation of intracellular calcium plays a critical role in cell death in various models of stroke, but the mechanism by which calcium produces cell death remains poorly understood.

In many types of neurodegeneration the intracellular calcium-dependent protease calpain is activated. In this study, the activation of calpain following irreversible occlusion of the middle cerebral artery in rats (MCA occlusion) was evaluated using the proteolysis of calpain substrates as a measure of calpain activity. Spectrin, a cytoskeletal protein which is prevalent in neurons, was extensively cleaved following MCA occlusion. The spectrin breakdown products observed were the same as those observed following in vitro cleavage of spectrin by calpain, indicating that calpain is the major protease acting on spectrin during the development of the infarcts. Spectrin cleavage was localized to regions which were infarcted as judged by TTC staining. The time course of the cleavage of spectrin followed closely the development of the infarct. Unlike global ischemia models, spectrin breakdown was not restricted to brain areas sensitive to excitotoxicity.

Administration of calpain inhibitors reduced the size of the infarct by 30% as judged by TTC staining 24 hours after occlusion. Inhibitors were effective when given intravenously beginning immediately after the occlusion. Taken together, these data argue that calpain activation plays a key role in the neurodegeneration following focal ischemia and stroke, and that inhibition of calpain provides a potentially powerful means of protecting neurons from ischemic damage.

S 202 STRUCTURAL STUDIES OF PROTEINS INVOLVED IN THE UBIQUITIN-MEDIATED PROTEOLYSIS SYSTEM: CRYSTAL STRUCTURES OF A UBIQUITIN CARRIER PROTEIN (E2) AND A DI-UBIQUITIN CONJUGATE, William J. Cook & Leigh C. Jeffrey, Center for Macromolecular Crystallography, University of Alabama at Birmingham, Birmingham, AL 35294; Michael L. Sullivan & Rick D. Vierstra, University of Wisconsin-Madison, Madison, WI 53706; Z. Chen & Cecile M. Pickart, University of Buffalo, Buffalo, NY 14214. The structure of a recombinant ubiquitin carrier protein (E2) from the plant *Arabidopsis thaliana* has been determined using multiple-isomorphous-replacement techniques and refined at 2.1 Å resolution. E2 is an α/β protein, with 4 α-helices and a 4 stranded antiparallel ß-sheet. There are long amphipathic α-helices at the N- and C-termini. The active site cysteine residue is in a small cleft on the opposite side of the molecule from the end of the C-terminus. The structure suggests that the first 150 residues of all E2s have a common tertiary structure, and various C-terminal extensions probably extend away from the core. Using another E2 that catalyzes multi-ubiquitin chain synthesis from purified ubiquitin, a di-ubiquitin conjugate has been synthesized and crystallized. These two ubiquitin molecules are connected through an isopeptide bond involving Lys48 of one ubiquitin and the C-terminal glycine residue (Gly76) of the other ubiquitin. The structure of this conjugate, which was determined by using molecular replacement techniques, has also been refined at 2.1 Å resolution. The conjugate shows pseudo-twofold symmetry, and the individual ubiquitin moieties are quite similar to native ubiquitin. Small hydrophobic patches on the surfaces of the two ubiquitin moieties, consisting of Leu8, Ile44, and Val70, form a hydrophobic core in the conjugate.

S 203 1-METHYL-4-PHENYLPYRIDINIUM (MPP+) INDUCES APOPTOTIC CELL DEATH IN NEURONS, Bruno Dipasquale, Ann M. Marini [1] and Richard J. Youle, Biochemistry Section, Surgical Neurology Branch, National Institute of Neurological Disorders and Stroke, and Clinical Neurosciences Branch[1], National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20892-0010.

Apoptotic cell death is a process of active self-destruction characterized by internucleosomal DNA fragmentation that has been described in corticosteroid-treated thymocytes as well as in several physiopathological conditions of the lymphoid system. In the nervous system programmed cell death activation leading to apoptosis has been observed during nematode development. We have observed that the neurotoxic agent 1-methyl-4-phenylpyridinium (MPP^+) induces apoptotic cell death in cultured cerebellar granule cells. Nuclear refractility changes characteristic of apoptosis were observed under Nomarski optics in granule cells after four days of treatment with MPP^+. We observed a 30% increase of apoptotic cells compared to control cultures. Surprisingly, a proportion of untreated neurons (~20%) showed morphological signs of apoptosis. Cycloheximide, a protein synthesis inhibitor, decreased the amount of apoptotic death in MPP^+-treated as well as in untreated granule cells demonstrating the requirement of new gene expression. Agarose gel electrophoresis of DNA extracted by granule cells showed a more pronounced internucleosomal degradation in MPP^+-treated cells. Cycloheximide blocked DNA fragmentation as well as the apoptotic refractility observed under Nomarski optics. Granule cells maintained in culture for a prolonged period were more sensitive to the toxic effect of MPP^+ and also showed a progressively increased number of apoptotic cells in the untreated cultures. These experimental observations suggest that apoptosis is an important pathway of cell death in the nervous system and that neurodegenerative disorders and possibly Parkinson's disease may result from the inappropriate activation of programmed cell death.

S 204 CALPAIN ACTIVATION FOLLOWING GLOBAL ISCHEMIA, Jill E. Foreman, Stephen Espitia, Juan Estrada, David Lutz, Reginald L. Dean, and Raymond T. Bartus, Cortex Pharmaceuticals, Inc., Irvine, Ca. 92718

Brain ischemia causes the death of selected cell populations within specific regions. The molecular mechanism by which ischemia causes the selective and delayed cell death is currently an active area of investigation in many laboratories, and recent work has established that several mechanisms, both extracellular and intracellular, contribute to an elevation of intracellular calcium which then sets in motion intracellular events leading to cell death. However, the critical intracellular events which follow the elevation of calcium and contribute to the delayed cell death, in which cells appear normal for 24-48 hours following ischemia, have not been established.

The activation of the intracellular protease calpain is one potential mechanism of cell death following ischemia. In this study, the proteolysis of calpain substrates, including the cytoskeletal protein spectrin, was used as a measure of calpain activity in situ. Spectrin proteolysis was detected following global forebrain ischemia in gerbils, and was greatest during the delayed phase of cell death in the hippocampus. Spectrin cleavage was correlated to the degree of cell damage observed histologically.

In the rat global forebrain ischemia model, spectrin proteolysis increased at 24 hrs post-occlusion, prior to observable cell damage using the Nissl stain. Electrophysiological analysis reveals that the slope and amplitude of the action potentials in the hippocampus are not altered until this time. As in the gerbil, the proteolysis and electrophysiological alterations were geatest during the delayed phase of cell death (48-72 hrs post-occlusion). Proteolysis is observed only in the areas where cell death is observed histologically. These observations suggest that calpain activation is an early event in the process of cell death, and thus may be a causative factor in the degeneration following ischemia.

S 205 PUTATIVE TAU ISOFORMS, SPECIFIC FOR PAIRED HELICAL FILAMENTS IN ALZHEIMER'S DISEASE, HAVE A DEVELOPMENTAL ROLE IN ESTABLISHING AXON POLARITY -- NOT IN CELL DEATH. W.L. Klein and W. Pope. Northwestern University Institute for Neuroscience, Evanston, Ill 60208 PHF-1 is a monoclonal antibody that recognizes phosphorylated tau in paired helical filaments of AD brain tissue; PHF-tau is totally absent from age-matched controls but immunoblots show it to be present in the developing human brain (Greenberg & Davies, in press). A key question is why PHF-tau is present in both developing and degenerating brains: Is this molecule intrinsically linked to nerve cell death, or is it linked to a specific state of nerve cell differentation, recapitulated in AD? Studies here have been done with the chicken CNS, a convenient and widely used model for developmental analysis. Immunoblots of chicken CNS homogenates showed that PHF-1 recognizes 3 major proteins at molecular weights similar to those seen in humans. Immunoreactivity (IR) in embryonic brain sections was abundant, unlike Alz-50 IR, which was sparse and sporadic. In retina, PHF-1 IR was highly selective for ganglion cells and did not correlate with cell death: IR was present at least two days before onset of ganglion cell degeneration, was very robust after cell death was finished, and occurred in most if not all ganglion cells. Because ganglion cells are the only long-axon neurons in retina, and because optic fiber axons were heavily labeled, the relationship of PHF-1 IR to axogenesis was explored, using cell culture. A striking feature of IR in culture was that differentiating ganglion cells showed localization of PHF-1 IR to a single neurite. Neuritic polarity for PHF-1 IR occurred when polyclonal tau IR and anti-MAP2 IR showed no polarity. Precocious polarity for the PHF-1 form of tau also was seen in situ, suggesting that this tau form is linked to the initial creation of axonal polarity. PHF-1 IR is absent from mature axons, so its specific role must be transient. The main point from these results is that a subset of tau proteins, highly specific for paired helical filaments of adult degenerating neurons, is linked in developing neurons to axonal outgrowth, not to cell death. This link to axonal outgrowth supports the hypothesis that degenerating neurons in AD are attempting to undergo neuritic regeneration, albeit abortively.

S 206 ALTERATIONS IN GLUTAMATE RECEPTOR mRNA EXPRESSION IN MUTANT HAN-WISTAR RATS: A MODEL FOR EXCITOTOXICITY, Jody E. Margulies, Randy W. Cohen, Michael S. Levine and Joseph B. Watson, Mental Retardation Research Center and Department of Psychiatry and Biobehavioral Sciences, UCLA, Los Angeles, CA 90024. A mutant strain of the Han-Wistar (HW) rat carries an autosomal recessive gene for spastic paresis which is characterized by the postnatal development of ataxia, tremor and hindlimb rigidity. The neuropathology of the HW mutant rat is represented by progressive degeneration of Purkinje cells in the cerebellum and CA3 pyramidal cells in the hippocampus. Previous voltage-clamp assays indicated that Xenopus oocytes injected with poly $(A)^+$ RNA isolated from the cerebellum of mutant rats displayed increased responses to glutamate and kainate [Cohen, et al., (1991) Mol. Brain Res. 11:27-36]. These findings suggested that the cerebellar degeneration may be induced by glutamate excitotoxicity. Molecular and electrophysiological approaches were used to investigate the role of glutamate receptors in this disorder and to determine if mRNA expression of different glutamate receptor subunits changes in developing mutant cerebellum. Northern blot analysis revealed differential expression of glutamate receptor subtype mRNAs (GluR1-5; original cDNAs obtained from S. Heinemann and M. Hollmann) in mutant cerebellum relative to controls in the two age groups studied (postnatal 30-35 days and 50-55 days). Companion voltage-clamp experiments in Xenopus oocytes injected with RNA from the 50-55 day mutants showed an enhanced current response to glutamate, suggesting that changes occur in the functional expression of glutamate receptors at an age corresponding to cerebellar degeneration. Based on these findings, we propose that combinatorial expression of glutamate receptors may be predictive of mutant HW rat neuronal degeneration and altered function of cerebellar glutamate receptors.

S 207 SULFATED GLYCOPROTEIN 2 (SGP-2): A NEW MOLECULAR MARKER FOR NEURODEGENERATION AND REGENERATION. P.C. May, P. Robison, K. Fuson, B. Smaltstig, D. Stephenson and J. Clemens, CNS and Molecular Biology Research, Lilly Research Labs, Eli Lilly and Co., Indianapolis, IN 46285

Sulfated glycoprotein-2 (SGP-2), is the rodent homologue to pADHC-9, a human hippocampal cDNA clone elevated in Alzheimer's disease (AD) [May et. al, 1990, Neuron 5:831]. Understanding the function of SGP-2/pADHC-9 in brain may point to novel mechanisms of neurodegeneration ongoing in AD. Here I summarize the results from several neuronal lesion paradigms used to explore the in vivo regulation of SGP-2. Kainate lesions of the hippocampus results in degeneration of intrinsic CA3/CA4 pyramidal neurons and is accompanied by 2-4 fold increases in SGP-2 RNA and protein levels. Deafferentation of the hippocampus by lesioning the perforant pathway from the entorhinal cortex also results in increased hippocampal SGP-2 expression, limited to areas undergoing active sprouting and reactive synaptogenesis (hilus and dentate gyrus molecular layer). Increased expression of SGP-2 after neuronal injury is not limited to the hippocampus. Marked increases in SGP-2 protein also occur in the degenerating caudate nucleus, as well as hippocampus, 3 days after transient global ischemia. These data suggest that the increased expression of SGP-2 is a generalized response to neuronal injury and that animal lesion paradigms should be useful for elucidating the function of SGP2/pADHC-9 in brain, particularly in recovery from neuronal injury.

S 208 PROLIFERATION AND SURVIVAL OF HIPPOCAMPAL NEURONS IN CULTURE STIMULATED BY NEUROTROPHIN-3 AND bFGF, Fukuichi Ohsawa, Timothy L. Denton, Beat Knusel, and Franz Hefti, Division of Neurogerontology, Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

Neuronal proliferation, survival and differentiation is affected by various growth factors. Many growth factors, including members of the neurotrophin family, are expressed at high levels in the developing and adult hippocampus. To identify populations of neurons responsive to individual growth factors and to characterize their actions, we studied the effects of neurotrophins and fibroblast growth factors on primary cultures of fetal rat hippocampal cells.

Dissociated cells from E15-20 rat hippocampi were cultured in serum-free L-15/N2 medium. Neurotrophins (NGF, BDNF, NT-3) and bFGF were present during the entire culture time. After 7 days in vitro, the number of surviving cells was measured by quantifying the transformation of MTT into MTT formazan, which is catalyzed by living cells. bFGF and NT-3 elevated the number of cells present after 7 days in vitro. For bFGF this effect was evident with cultures prepared from E15 to 17 but not E18 and 20, for NT-3 it was only observed on E15 cultures. No comparable actions occurred with NGF and BDNF. With both bFGF and NT-3 the number of surviving cells increased during the culture time, indicating a stimulation of cell proliferation. Morphological and immunohistochemical analysis for neuronal and glial marker proteins identified the surviving cells as neurons. In cultures from E15 hippocampi, neuronal survival was pronounced in absence of growth factors, whereas very few cells survived under control conditions in cultures prepared from later stages. Neuron survival in E15 cultures was inhibited by the neurotrophin antagonist K-252b. The result suggest that hippocampal neurons at E15 are maintained by neurotrophins produced by autocrine mechanisms whereas, at later stages, exogenous bFGF is able to stimulate neuronal proliferation and differentiation.

S 209 ALZ50 RECOGNIZES A NON-PHOSPHORYLATED EPITOPE OF TAU IN NORMAL AND ALZHEIMER'S DISEASE CEREBRAL CORTEX. David Parkinson, Dennis Q. McManus and John C. Morris. Departments of Cell Biology and Physiology, and Neurology, and Pathology (Neuropathology), Washington University Medical School, St. Louis MO 63110.

The relationship between the ALZ50 antigen and tau was studied by western blotting of a tau-enriched fraction prepared from samples of cerebral cortex obtained at autopsy. Donors were apparently normal individuals ranging in age from 32 to 86 years and Alzheimer's disease (AD) patients from an extensively studied sample that had been followed prospectively.

ALZ50 antigen was detected as four major bands of 48-56kD. The same pattern of staining was seen in both normal and AD patients, regardless of age. The intensity of ALZ50 staining in AD brain appeared to be in the range of normal brain samples. Treatment of the samples with alkaline phosphatase increased the mobility of ALZ50-positive bands, but did not affect the intensity of staining.

The same samples were immunoblotted with a polyclonal antibody to chicken brain tau. In the normal samples, five or six tau bands were stained and a sub-set of these co-migrated with the ALZ50-positive bands. This pattern was also seen in the AD samples except that additional tau-positive bands were seen at lower mobility. These additional bands did not appear to react with ALZ50. The staining intensity of tau-positive bands from AD brain was in the same range as normal brain samples. After treatment with alkaline phosphatase, the number of tau-positive bands was reduced to four bands of higher mobility that co-migrated with the ALZ50-positive bands.

These results show that the ALZ50 antigen is expressed in both normal and AD cerebral cortex and is probably on a sub-set of the tau proteins. Furthermore, binding of ALZ50 to its epitope does not appear to be dependent on phosphorylation.

S 210 PHOSPHORYLATION OF TAU PROTEIN BY cAMP-DEPENDENT PROTEIN KINASE: IDENTIFICATION OF PHOSPHORYLATION SITES AND EFFECT ON TAU FUNCTION, Clay W Scott, Francis H. Chow, Joseph L. Herman, Claudia B. Caputo and Russell C. Spreen, ICI Pharmaceuticals Group, ICI Americas, Wilmington, DE 19897.

Neurofibrillary tangles represent a classical intracellular hallmark of Alzheimer's pathology. Numerous studies have shown that tau protein is an integral component of paired helical filaments (PHF), the repeating subunit comprising neurofibrillary tangles. Studies with phosphate-dependent antibodies have shown that tau protein is abnormally phosphorylated when localized to the PHF. However, the actual sites of phosphorylation on normal and PHF-tau are not known, and the effect of abnormal phosphorylation on tau function has not been determined. We have begun to map the sites on human recombinant tau that are phosphorylated in vitro using various purified protein kinases, and have examined the effect of these phosphorylations on tau function. Phosphorylation sites were determined by digesting phospho tau with proteases, separating the peptides by reversed phase HPLC, and analyzing the isolated peptides by FAB-MS. This approach, combined with Edman degradation of isolated peptides and phosphoamino acid analysis of 32P-labelled samples, revealed that ser 214, 324 and 356 (based on the amino acid sequence of the longest isoform) represent phosphorylation sites for cAMP-dependent protein kinase (PKA). Phospho and nonphospho recombinant tau were analyzed in a microtubule bundling assay. Both samples showed similar dose-dependent bundling of microtubules, indicating that phosphorylation by PKA did not affect the ability of tau to crosslink microtubules.

S 211 REGULATION OF PROTEASE NEXIN-1 SYNTHESIS AND SECRETION IN CULTURED CELLS BY INJURY-RELATED FACTORS, Patrick J. Vaughan, Denis Guttridge and Dennis D. Cunningham, Department of Microbiology and Molecular Genetics,University of California, Irvine, CA 92717.

Protease nexin-1 (PN-1) is a 43kDa protease inhibitor that can inhibit several serine proteases, although its physiological target is probably thrombin. PN-1 is identical to the glial-derived neurite promoting factor or glial-derived nexin (GDN). This neurite promoting activity of PN-1 is mediated through its ability to inhibit thrombin, a protease which can retract processes on both neurons and astrocytes. It is interesting in this respect that much of the PN-1 in human brain occurs around blood vessels where it may play a protective role against extravasated thrombin following injury within the brain. Other studies also indicate that PN-1 may play key roles after injury and may be important in certain pathological conditions. Thus following lesion of the sciatic nerve in rats, PN-1 levels are increased in distal regions of the nerve. In addition PN-1 is decreased in the hippocampus and cortical regions of Alzheimer's disease brain compared to age-matched controls. In the present studies we have examined the synthesis and secretion of PN-1 by factors known to be produced after injury and in inflammatory processes. We have found that PN-1 synthesis is dramatically stimulated by four factors, Interleukin-1, Transforming Growth Factor-ß, Tumor Necrosis Factor α, and Platelet-derived Growth Factor, that play key roles in the wound repair process. In addition one factor, Dexamethasone, which is known to inhibit wound repair mechanisms, downregulates the synthesis of PN-1. Collectively these data strongly suggest that following injury PN-1 may play an important protective role in the brain.

S 212 STIMULATION OF PHOSPHATIDYLINOSITOL-BREAKDOWN IN CORTICAL CULTURES BY THE NEUROTROPHINS *BDNF* AND *NT-3* BUT NOT *NGF*, Hans Rudolf Widmer, Beat Knüsel, and Franz F. Hefti, Division of Neurogerontology, Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089

Phosphatidylinositol (PI) breakdown represents a powerful system participating in the transduction mechanism of some neurotransmitters and growth factors and producing two second messengers, diacylglycerol and inositol triphosphates. One family of neurotrophic factors, the neurotrophins, play an important role during brain development, adult function and, possibly, in neurological disorders. Three members of this family are present in the adult central nervous system: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3). The NGF-mediated transformation of PC12 neuroblastoma cells into neuron-like cells is preceded by a rapid stimulation of PI-breakdown, however, it is not known whether PI breakdown mediates actions of other members of the neurotrophin family. We present initial evidence, that BDNF and NT-3 stimulate PI-breakdown of fetal rat brain neurons in primary cell cultures. PI was labeled by incubating cultures with [^{3}H]myo-inositol. BDNF and NT-3, but not NGF, elevated the levels of labeled inositol phosphates within 10-15 min after addition to the cultures with ED_{50} values of 20-60 nM. Atropine, which inhibited the carbachol stimulated PI-breakdown in these cultures, failed to inhibit the action of neurotrophins. K252b, which selectively blocks neurotrophin actions by inhibiting trk-type receptor proteins, prevented the PI-breakdown mediated by BDNF and NT-3. The actions of BDNF and NT-3 probably are neuron specific since no stimulation was detected in cultures of non-neuronal cells. The study suggests that rapid and specific induction of PI-breakdown is involved in the signal transduction of BDNF and NT-3. Furthermore, the findings provide evidence that cortical neurons are functionally responsive to BDNF and NT-3 during early development.

S 213 DIVERSE EFFECTS OF NEUROMUSCULAR BLOCKADE ON LUMBAR MOTONEURONS AT DIFFERENT STAGES OF DEVELOPMENT IN CHICK EMBRYO. QIN-WEI YIN AND SHEPU XUE$. Dept. of Neurobiology and Anatomy, Bowman Gray Sch. of Medicine. NC. and $Dept. of Cell biology. Peking Union Medical college and Chinese Academy of medical science.

Although previous studies have revealed that both presynaptic and postsynaptic blockade of neuromuscular activity during the period of natural-occurring motoneuron death in the chick embryo prevents motoneurons from normal cell death, there is a lack of investigation on the effect of neuromuscular blockade on motoneurons after the normal cell death period. Hemicholinium-3 (HC-3) bromide was dissolved in 200ul of 0.9% NaCL and administered directly onto the highly vascularized chorioallantoic membrane. Embryos given HC-3 per day from embryonic day (E)5 to E6, E9, E13 and E17 were sacrificed on E7, E10, E14 and E18. The dose of HC-3 varied from 1-3mg depending on age. One to two days before sacrifice, a choleragen subunit B conjugated HRP (CB-HRP) was used to retrogradely label the somas and dendrites of motoneurons innervating hindlimb muscles. On E7, E10 or E14, there was an increase in length and distribution density of motoneuron dendrites in lumbar cord of chick embryo following the application of HC-3. In addition, it was observed that some labelled motoneuron processes project into the central canal, which is absent in normal cases. However, surprisingly, by E18 about 70% of motoneurons die, numerous pyknotic cells were detected, the size of motoneuron soma becomes smaller (60% of that in the normal), dendrites degenerate significantly (about 1/7 of normal length) and distribution area of dendrites decreases dramatically. Accordingly, the present data suggest that putative neurotrophic agents from muscles can regulate survival, growth and differentiation of motoneurons of lumbar cord. So it appears that long-term blocking of normal motoneuron-target communication may result in a decrease or cessation in release of trophic agents from muscle cells, which, in turn, could arrest the development of the motoneurons.

Animal Models and Therapeutic Strategies

S 300 DIFFERENTIAL STIMULATION OF DOPAMINERGIC NEURON DEVELOPMENT BY BRAIN-DERIVED NEUROTROPHIC FACTOR IN VITRO. Klaus D. Beck, Beat Knüsel and Franz Hefti, Andrus Gerontology Center, University of Southern California, Los Angeles CA 90089-0191.

Brain-derived neurotrophic factor (BDNF) supports survival and differentiation of basal forebrain cholinergic neurons in vitro and in vivo, similar to NGF but with lower potency (Alderson et al. 1990, Knüsel et al. 1991). BDNF, in contrast to NGF, also increases survival and promotes differentiation of dopaminergic neurons in mesencephalic cell cultures (Hyman et al. 1991, Knüsel et al. 1991). To provide a detailed analysis of the nature of trophic actions of BDNF on dopaminergic neurons, we studied distinct parameters of dopaminergic differentiation in vitro. Exposure of mesencephalic cell cultures to BDNF for 5 days resulted in an approximately twofold increase of [^{3}H]-dopamine uptake, while tyrosine hydroxylase (TH) activity was only slightly elevated. The number of TH-immunopositive cell was increased to 135% of controls. The number of primary neurites extending from these cells was significantly increased. To analyze potential effects of BDNF on the transcription of cell-specific genes, expression of TH mRNA was studied with northern blotting and in situ hybridization techniques. No increase could be detected following exposure to BDNF. Furthermore, BDNF was tested in the MPP^+ model of selective dopaminergic neurotoxicity. Pretreatment of cultures for 3 days protected dopaminergic neurons against cell death induced by moderate concentrations of MPP^+ and promoted recovery of DA-uptake.

These data indicate that various molecular and cellular parameters reflecting DA cell functions are differentially affected by BDNF.

S 301 BIOCHEMICALLY DISTINCT ISOFORMS OF PrP^{TME} ISOLATED FROM TWO STRAINS OF TRANSMISSIBLE MINK ENCEPHALOPATHY, Richard A. Bessen and Richard F. Marsh, Department of Veterinary Science, University of Wisconsin-Madison, Madison, WI 53706

Transmissible mink encephalopathy (TME) is a rare, neurodegenerative scrapie-like disease of ranch-raised mink. Experimental transmission of Stetsonville TME to outbred Syrian hamsters resulted in two distinct biological syndromes, called HYPER (HY) and DROWSY (DY), that emerged by third hamster passage. The HY and DY syndromes differed with respect to clinical disease (hyperexcitability and ataxia vs. lethargy), incubation period (65±1 days vs. 168±1 days), brain titer ($10^{9.5}$ LD_{50}/gm of tissue vs. $10^{7.4}$ LD_{50}/gm of tissue), brain lesions (absence or presence of large vacuoles adjacent to CA2 region of the hippocampus), and interspecies transmission (nonpathogenic or pathogenic upon backpassage into mink). The presence of the HY and DY strains of agent, that retain their biological characteristics on repeated hamster passage, in the Stetsonville TME source requires that the informational molecule encoding these transmissible agents have the capacity to account for this biological diversity.

The prion hypothesis claims that the infectious agent causing TME is a posttranslationally modified host protein, called PrP^{TME}, that acquires amyloid-like properties. Purification and analysis of PrP from hamsters infected with the HY and DY TME strains revealed differences in PrP^{TME} sedimentation properties in N-lauroylsarcosine, sensitivity to digestion with proteinase K, and migration in polyacrylamide gels. Antigenic mapping of PrP^{TME} with antibodies made to synthetic peptides revealed strain-specific differences in immunoreactivity at the N-terminal end of proteinase K-treated PrP^{TME} at amino acid residues 90 to 103. These findings indicate that the amyloid-like PrP^{TME} from the two agent strains, although originating from the same host, differ in composition and/or conformation. We are attempting to identify the physicochemical differences between HY and DY PrP^{TME} in order to understand how this protein becomes posttranslationally modified, and the potential of PrP^{TME} to induce neurodegeneration in a strain-specific manner.

S 302 RETROVIRUS-INDUCED SPONGIFORM ENCEPHALOMYELOPATHY: HOST AND VIRAL DETERMINANTS OF THE LENGTH OF THE INCUBATION PERIOD, Markus Czub, Frank McAtee and John Portis, Laboratory of Persistent Viral Diseases, NIAID, Hamilton Montana. A molecular clone of the wild mouse ecotropic retrovirus, CasBrE, causes a spongiform neurodegenerative disease with a long incubation period of ≥ 6 months. However, when the 3' *pol* and *env* gene of this virus were introduced into the genome of a strain of F-MuLV, the resulting chimeric virus, $FrCas^E$, caused accelerated disease with an incubation period of only 16 days. Induction of the disease is dependent on inoculation of the virus into neonates. In the current study we have identified both host and viral factors which appear to be important determinants of incubation period. The length of the incubation period is a function of the level CNS infection. To examine the possible function of viral sequences which influence the level of CNS infection, a group of chimeric viruses was utilized which infect the CNS at different levels and cause disease with different incubation periods. The relative level of CNS infection appeared to be a function the viremia kinetics during the first 10 days postinoculation. Viruses which reached peak titers between 4 and 6 days postinoculation infected the CNS at high levels, slower viruses infected the CNS at lower levels. Among the chimeric viruses used in this study, early viremia kinetics was determined by sequences within the 5' leader sequence of the viral genome. We have previously shown that the CNS is susceptible to infection only during the perinatal period (J. Virol 65: 2539-2544, 1991). We have extended this study and found that the susceptibility of the CNS to infection wanes progressively or gradually as a function of age, the restriction being complete by the age of 12-14 days. Taken together these results suggest that the relative levels of CNS infection and, inturn, the length of the incubation period is determined very early in life, and appears to be a function of a dynamic relationship between the kinetics of virus replication in the periphery and a progressively developing restriction to virus replication in the CNS.

S 303 THE EVOLUTION OF SPONGIFORM LESIONS INDUCED BY A NEUROTROPIC MURINE RETROVIRUS, Stefanie Czub, Markus Czub and John Portis, Laboratory of Persistent Viral Diseases, NIAID Hamilton Montana 59840. The chimeric murine retrovirus $FrCas^E$, which harbors the envelope gene of the wild mouse retrovirus CasBrE, induces fatal neurodegenerative disease after neonatal inoculation with an incubation period of only 14-16 days. The disease is characterized by a highly predictable sequence of events. The virus first replicates in peripheral organs producing a viremia which peaks at 4 days postinoculation. Infection of the CNS is first detected 4 days postinoculation. The first signs of spongiform degeneration are seen at 10 days postinoculation followed by clinical disease (tremor and paralysis) at 14-16 days postinoculation. The lesions induced by this virus consist of initial swelling of post-synaptic processes and are <u>not</u> associated with neuronal dropout, gliosis, or inflammation. The distribution of $FrCas^E$ within the CNS has recently been described by Lynch et al (Neuron 7: 365-379, 1991). Infected microglial cells are found in the vacinity of the affected neurons, but the neurons themselves appear not to be infected. This has raised the possibility that a locally produced neurotoxin, either of viral or microglial origin, may be involved in the pathogenesis of this disease. Interestingly, there are certain populations of neurons which <u>are</u> productively infected by this virus. These include the granule neurons of the cerebellar cortex. In the current study we have mapped in detail the evolution of the lesion profile in this disease as a function of time after inoculation, and the results suggest an alternative explanation of the neuropathology induced by this virus. The distribution of lesions is stereotypic, involving only nuclei of the motor system from the spinal cord caudally to the motor cortex rostrally. The first signs of vacuolar degeneration, seen 10 days postinoculation, appear simultaneously in all of these areas. The distribuion of the lesions within the CNS does not change thereafter, but the cytopathology becomes more extensive within the areas already affected. Finally, the lesion profile indicates that, without exception, all affected areas have in common, either mono, di or trisynaptic connections to the Purkinje cells of the cerebellar cortex. These observations suggest the possibility of transsynaptic degeneration and further suggest a possible role of cerebellar cortical dysfunction in the pathogenesis of this disease.

S 304 DIFFERENCES IN GLYCOSILIC MOIETY AND FATTY ACID COMPOSITION OF GANGLIOSIDES IN SCRAPIE-INFECTED HAMSTER BRAINS, Alessandro Di Martino[1], Jiri Safar[1], Rachel L. Sherman[1], Norman Salem Jr[2] and Clarence J. Gibbs Jr[1], Laboratory of CNS Studies, NINDS [1], and Laboratory of Membrane Biochemistry and Biophysics, NIAAA [2], National Institutes of Health, Bethesda, MD, 20892.

Although ganglioside composition in brain tissue in natural or experimentally-induced spongiform encephalopathies has already been studied, no quantitative data have been obtained for hamsters infected with the adapted 263K strain of scrapie agent, which constitutes the most widely used animal model for studies on unconventional agents. Additionally, all previous experimental data obtained in primates and rodents do not appear to be consistent. While preliminary results have indicated that significant changes in composition occurred in brains recovered from terminally-ill infected hamsters, the present work aimed to determine if the differences were specific, reproducible, and statistically significant within single animals. Results indicated that gangliosides showed little changes in their major components in scrapie-infected animals. However, an increased number of new species bearing alkali-labile modifications of the sialic acid moiety appeared, together with differences in ceramide composition, including a general decrease of saturated fatty acids. This indicates that these specific components of neuronal membranes undergo abnormal biochemical modifications at the terminal stage of scrapie. In addition, the animal strain we employed demonstrated a significant polymorphism in brain ganglioside composition in both normal and scrapie-infected animals. No significant changes in incubation time, clinical development or pathologic features of scrapie were associated with this specific finding.

S 305 CALPAIN INHIBITORS AS THERAPEUTICS FOR STROKE AND ISCHEMIA, David D. Eveleth, Hau Ton, Reginald L. Dean, Stephen Espitia, Kathleen P. Cavanaugh, Jill Miotke, Deborah Henson-Snow, David Lutz, Sue Tatarewicz, Brian Miyazaki, Juan Estrada, James C. Powers, and Raymond T. Bartus, Cortex Pharmaceuticals, Inc., Irvine, CA 92718

The elevation of intracellular calcium in nerve cells following a wide variety of insults is thought to be a common pathway leading to cell death. The role of intracellular calcium in excitotoxic cell death is well established. However, the specific events triggered by calcium which induce cell death are not well understood. One event which may play a causative role in calcium-mediated cell death is the activation of the protease calpain. If calpain is involved, then inhibition of calpain should reduce cell death following excitotoxicity or ischemia.

Calpain inhibitors promoted the recovery of evoked potentials in hippocampal slices following hypoxia. The increased recovery of evoked potentials was observed after administration of several different inhibitors of differing chemical classes. This increase was dose-dependent and also required incubation of the slices with inhibitor prior to hypoxia.

In the rat four vessel occlusion model of transient global ischemia, administration of calpain inhibitors directly into the CA1 region of the hippocampus caused a significant increase in the number of surviving cells in the pyramidal layer. The rescue of pyramidal cells was observed even when inhibitor was administered after the end of the ischemic period. Intravenous administration of inhibitor after reperfusion also resulted in a significant increase in the number of surviving pyramidal cells.

These observations indicate that the inhibition of calpain reduces neural damage following ischemia. Activation of calpain is therefore a part of the mechanism leading from ischemia to pathology, and inhibition of calpain may be a useful therapeutic approach.

S 306 NEURODEGENERATIVE AND IMMUNOCYTOCHEMICAL CHANGES OF CHRONIC WASTING DISEASE IN CAPTIVE MULE DEER, Guiroy DC, Williams ES[†], Gajdusek DC, NIH, Bethesda, Maryland 20892, [†]University of Wyoming, Laramie, Wyoming 82070

Chronic wasting disease (CWD) is a rapidly progressive, uniformly fatal neurological disorder affecting captive mule deer and Rocky Mountain elk. To determine if the neurodegenerative and immunocytochemical changes in CWD were similar to those found in other diseases belonging to the subacute spongiform encephalopathies, we studied brain tissues from nine CWD-affected mule deer and 2 neurologically normal mule deer. Neuropathological changes consisted of chromatolytic, ballooned neurons with eccentric nuclei, neuronal satellitosis, single and multiple intraneuronal vacuolations, status spongiosus of the neuropil and astrocytic hyperplasia and hypertrophy. Purkinje cells, in particular, showed varying degrees of cytoplasmic vacuolations and fenestrations, and axonal vacuolation. Loss of Purkinje cells and replacement with vacuoles, reduced numbers of granule cells, numerous axonal spheroids in the granular and pyramidal layers were also found. Immunocytochemical findings included axonal spheroids immunoreactive to phosphorylated neurofilament-H, intracytoplasmic accumulation of neurofilament-H, astrocytic hyperplasia and hypertrophy as confirmed by immunoreactivity to glial fibrillary acidic protein, activated and resting microglia immunoreactive to antibody against ferritin. Similar neurodegenerative changes have been observed in natural and experimental kuru and Creutzfeldt-Jakob disease, further corroborating that chronic wasting disease belongs to the subacute spongiform encephalopathies. Studies are under way to determine if the scrapie precursor protein gene is also present in chronic wasting disease.

S 307 High level Expression of the Kunitz-type Protease Inhibitor Domain of the Alzheimer's Amyloid Protein Precursor/Protease Nexin-2 in Pichia Pastoris.

Harpold, M.M.[1], Siegel, R.[1], Comer, W.T.[1], Vedvick, T.[1], Raschke, W.C.[1], Van Nostrand, W.E.[2] and Wagner, S.L.[1]

[1]The Salk Institute Biotechnology/Industrial Associates, Inc., (SIBIA) La Jolla, CA. 92037 and [2]Department of Microbiology and Molecular Genetics, University of California, Irvine, CA. 92717.

The methylotrophic industrial yeast, P. pastoris was used as a host system for large scale production of the Kunitz-type protease inhibitor domain of APP751/PN-2. In addition to the 57 amino acid Kunitz-type inhibitory domain, the expression product contained an additional 4 amino acid residue which corresponds precisely to amino acids 285-288 of APP751/PN-2. Using this type of expression system we were able to generate yields of greater than 1.0 gram/liter. The secreted 61 amino acid peptide was purified to homogeneity, sequenced and was fully active in terms of its ability to inhibit trypsin, chymotrypsin, epidermal growth factor binding protein and coagulation factor XIa. The protease inhibitory properties of this peptide were highly similar to those of PN-2. Although heparin is capable of augmenting the inactivation of factor XIa by PN-2, it had no effect on the inactivation of factor XIa by this 61 amino acid peptide. This active human peptide should be useful in studying the physiological/pathophysiological mechanism(s) leading to amyloid deposition.

S 308 STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF THE MOUSE NERVE GROWTH FACTOR PROMOTER REGION. Eric K. Hoffman, Heide M. Wilcox, Kristin C. Hartpence, Michael E. Lewis and Susan Carswell. Cephalon, Inc. 145 Brandywine Parkway, West Chester, PA 19380. Nerve growth factor (NGF) expression can be induced by a variety of seemingly unrelated compounds, including: serum, agonists of beta adrenergic receptors, retinoic acid, IL-1 beta, catecholamines and catechol derivatives. Treatment of mouse L929 cells with these compounds leads to an increase in NGF mRNA, suggesting that induction is most likely occurring at the level of transcription. To gain insight into how transcription of this gene is regulated at the basal and induced levels, we have isolated and characterized the promoter region of the mouse NGF gene. Transient expression studies utilizing the bacterial chloramphenicol acetyltransferase (CAT) gene were used to initially localize DNA sequence elements important for NGF promoter function. Stepwise deletion of 5' sequences revealed the location of sequences critical to basal level expression. These deletion constructs were also used to study the specific DNA sequence elements in the NGF promoter region that are required for the induction of this gene by various compounds.

S 309 EARLY DETECTION OF UBIQUITIN-PROTEIN CONJUGATE IMMUNOREACTIVITY IN SCRAPIE-INFECTED MICE, Nigel Kenward*, Jill Fergusson*, Michael Landon*, James Hope†, Helen McDermott*, David McQuire‡, James Lowe‡ and R. John Mayer*, Departments of Biochemistry* and Pathology‡, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH, and AFRC and MRC Neuropathogenesis Unit†, Ogston Building, Edinburgh EH9 3JF, U.K.

Brains from mice infected with either the ME7 or the 87V strains of sheep scrapie were assessed for the presence of ubiquitin-protein conjugates at various stages of the disease process. We have previously described the pattern of immunochemical staining for ubiquitin-protein conjugates in the terminal stages of clinically-ill ME7- and 87V-infected mice (Lowe *et al.* J. Pathol. 162, 61, 1990). We now report that one of the three types of immunoreactive deposit that we saw in the end -stage animals is also visible in select brain areas as early as 28 days and 171 days post-infection in ME7- and 87V-infected animals, respectively. Thus, both groups of animals exhibited fine dot-like structures in the neuropil; this form of pathological feature preceded the appearance of spongiform change. As the disease progressed, there was an increase in the extent of the immunoreactive lesions with coarser, peri-karyonal deposits and, somewhat later, staining of the peripheral regions of plaques becoming apparent. As noted in our earlier study, the peri-karyonal immunoreactivity was associated with areas of vacuolation and therefore occurred more widely in the ME7-infected animals, while plaque-associated immunoreactivity was greater in those infected with the 87V strain of scrapie. In these two mouse models of the prion encephalopathies the ubiquitin system appears to be activated at early stages of the disease process. Our results show that ubiquitin conjugation is an important mechanism in the development of the abnormal features that characterise this disease and appears, therefore, to be involved intimately in its pathogenesis.

S 310 CHARACTERIZATION AND MANIPULATION OF YEAST ARTIFICIAL CHROMOSOMES CONTAINING THE HUMAN AMYLOID PRECURSOR PROTEIN GENE, Bruce T. Lamb and John D. Gearhart, Developmental Genetics Laboratory, Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, MD 21205

We have characterized nine different yeast artificial chromosomes (YACs) that contain human amyloid precursor protein (*APP*) sequences and vary in size from 150 Kb up to 1,000 Kb. As determined by extensive restriction analysis, the four largest YACs (650 Kb, two 850 Kb, and 1,000 Kb) contain the entire *APP* gene, including the promoter and all 18 exons. The five smaller YACs (150 Kb, two 200 Kb, 210 Kb, and 340 Kb), on the other hand, contain only exons 9 through 18. A restriction map of the *APP* genomic region using rare-cutting restriction enzymes has been generated which delineates the actual size of the *APP* gene. We have transferred two of the larger YACs containing the entire *APP* gene into yeast strain YPH857, for further manipulation. In this background, we have introduced a neomycin resistance (neo^r) expression cassette that is highly active in embryonic stem (ES) cells into one of the YACs. We are currently attempting to introduce a purified and concentrated YAC containing an entire human *APP* genomic copy and the neo^r cassette into ES cells and eventually into the germline of mice. The transfer of an entire human *APP* genomic copy into mice should yield the most faithful animal model for Alzheimer's disease as well as for the amyloidosis seen in Down Syndrome.

S 311 OVERCOMING THE DEVELOPMENTAL RESISTANCE TO NEUROVIRULENT MURINE RETROVIRUS INFECTION BY CNS WOUNDING: INFECTION OF MICROGLIA IN THE ABSENCE OF SIGNIFICANT SPONGIFORM DEGENERATION, William P. Lynch, John L. Portis, Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories, NIAID, NIH, Hamilton, MT 59840.

The chimeric wild mouse retrovirus, $FrCas^E$, causes rapid spongiform neurodegenerative disease within 16 days when inoculated into neonatal mice. Clinically, the disease is characterized initially by hind limb paralysis with associated tremor, progressing to decerebrate rigidity and ultimately results in death by day 25. The disease is clearly associated with the capacity of the virus to infect the CNS, as animals infected later than 10 days after birth are resistent to CNS infection even though they develop a significant peripheral viremia. Neonatal inoculation result in infection of three major cell types in the CNS: the granule neurons of the cerebellar cortex, endothelial cells and pericytes throughout the CNS, and ramified and ameboid microglial cells which are concentrated in the areas undergoing spongiform degeneration. Given the spatial relationship between the degenerating neurons and the infected microglia we endeavored to ask whether this infection alone could be responsible for the observed spongiform changes. To accomplish this we infected animals 10 days after birth and then 7 days later wounded the CNS by inserting a flame heated needle through the cortex and into the thalamus. Seven days after wounding the animals were examined for the presence of infected microglia associated with the wound site. Significant infection of both ramified and ameboid microglia cells was observed both along the needle track and in distal sites corresponding to areas of cellular migration. In the thalamus, an area where spongiform changes are readily observed in the neonatal model, little evidence of spongiform change could be detected as a result the local microglial infection. These data indicate that the resistance to CNS infection seen in 10 day old inoculated animals is probably a result of the failure of the virus to infect endothelial cells and therefore use them as a conduit to the CNS. In addition, these data indicate that the presence of infected microglia in the vicinity of susceptible neurons is not sufficient to cause spongiform degeneration. This may indicate that the neurons become developmentally resistent to the effects of the infected microglia or this may suggest the possibility that infected microglia are a result of the disease rather than a cause.

S 312 SEQUENCE AND METHYLATION IN THE β/A4 REGION OF THE RABBIT APP GENE, Maroun L.E., West R.L., Kotikalalpudi P. and Davidson J.S., Department of Medical Microbiology/Immunology, Southern Illinois University School of Medicine, Springfield, IL 62794-9230.

We have previously mapped the APP gene to rabbit chromosome #13 using in situ hybridization. A screen of a rabbit genomic λ library yielded two clones representing 18.6 kb of the β/A4 region of the rabbit gene.

The complete sequence of exons 16 and 17, which encode the β/A4 peptide, predicts that the rabbit peptides would be identical to the human and that a potential translational control consensus sequence in the mRNA is retained. There is a non-sense strand open reading frame for more than 100 amino acids in intron 15 and a simple sequence tandem repeat in intron 16.

Sequential probing of MspI/HpaII southern blots with individual exons has located a number of methylated CpG sites in this region which are carried on the λ clones. When probed with a combination of exons 15, 16, 17 and 18, the MspI banding pattern had four strong bands at 2.2, 3.0, 3.6 and 4.0 kb. The 3.0, 3.6 and 4.0 kb bands were replaced by larger bands in HpaII digests. The 3.0 kb band is absent when these filters are hybridized with a probe containing only exon 17.

Methylation patterns observed in sperm, and various tissue types, are being analyzed to determine if these sites are involved in de novo or imprinted methylation events.

S 313 ASSOCIATION BETWEEN THE ACCUMULATION OF PROTEINASE K- RESISTANT PRION PROTEIN AND INFECTIVITY IN MOUSE SPLEEN AND BRAIN, Race,R.E. and Darwin Ernst, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Rocky Mountain Laboratories, Hamilton, Montana 59840. The sequential accumulation of the protease resistant form (PrP-res), of an endogenous protein called prion protein (PrP) was compared to levels of scrapie infectivity in the spleens and brains of scrapie-infected mice at various times after infection. PrP-res was detected in mouse spleen by one week after infection, increased 65 fold by three weeks post infection but only 10-fold during the next seventeen weeks. This pattern was associated with infectivity which reached a maximum level and had plateaued by three weeks after inoculation. In mouse brain, PrP-res was not detected until approximately eight weeks after inoculation and then increased 200 fold during the next twelve weeks. In mouse brain infectivity increased 1000-10000 fold between 8 and 20 weeks postinoculation. Therefore, in mouse spleen PrP-res accumulation and infectivity appeared to be associated. However, the association was less apparent in mouse brain.

S 314 **TRANSGENIC MICE EXPRESSING β-AMYLOID PROTEIN OF ALZHEIMER'S DISEASE IN NON-NEURONAL CENTRAL NERVOUS SYSTEM CELLS**, Faheem A. Sandhu[1] and Sayeeda Zain[1,2], Department of Biochemistry[1] and Cancer Center[2], University of Rochester School of Medicine, Rochester, NY 14642

Senile plaques and cerebrovasculature of Alzheimer's disease (AD) contain abundant quantities of a small, insoluble protein, termed β-amyloid. *In vivo* studies of β-amyloid processing have been limited due partly to inadequate animal models for analysis. Contributions of both neuronal and glial cells to β-amyloid processing and deposition in AD are still unclear and need to be understood. We have used the JC viral early region promoter to direct the expression of the mRNA for the carboxyl 100 amino acids of the human β-amyloid protein specifically in the brains of transgenic mice (Sandhu FA, *et al., J. Biol. Chem.* **266**, 21331-21334, 1991). This promoter confers constitutive expression of the human β-amyloid transgene in non-neuronal cells of the central nervous system. Immunochemical and histochemical analysis of these mice will be discussed.

S 315 Co-Distribution of Protease Nexin-1 and Protease Nexin-2/APP_{sec} in Brains of Non-human Primates

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Protease nexin-1 (PN-1), a potent inhibitor of thrombin, and protease nexin-2 (PN-2), a potent inhibitor of coagulation factor XIa, were measured in various tissues of monkeys using monoclonal antibodies for either quantitative immunoblotting or ^{125}I-protease binding assays. Both PN-1 and PN-2 were much more abundant in brain than in any other tissues examined. PN-1 was found to be exclusively localized in brain. PN-2 was most abundant in brain, followed by testis and to a lesser extent kidney. Other tissues examined including spinal cord, heart, pancreas, spleen, liver, lung and muscle were essentially devoid of both PN-1 and PN-2. Within the brain, the levels of PN-1 and PN-2 were highest in the parietal cortex and lowest in the cerebellum and brainstem. The thalamus and striatum contained intermediate amounts of both proteins. Aged Cebus monkey cerebral cortical tissue contained slightly lower levels of PN-1 than did the middle-aged or young monkey tissue. The exclusive abundance of PN-1, and to a lesser extent PN-2, in brain suggests that these proteins may play a unique regulatory role in certain aspects of coagulation in the brain and that of this function may diminish with age.

Late Abstract

THE RELATIONSHIP BETWEEN THE gp75 AND THE gp140^{TRK} NERVE GROWTH FACTOR BINDING PROTEINS, T.W. Hepburn, K. Spiegel, R.J. Wyborski, H. LeVine and R.E. Davis, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105

Nerve growth factor (NGF) binds to two distinct proteins, the gp140^{trk} proto-oncogene product, a major signalling receptor for NGF, and the gp75 binding protein. The gp75 NGF binding protein lacks a cytoplasmic tyrosine kinase domain and does not appear to participate directly in the short-term signalling events consequent to the interaction of NGF with cell surface receptors. The gp75 binding protein, however, is regulated in important ways in nervous tissue. The interaction between these two NGF binding proteins is not known.

We have studied the differential regulation of gp140^{trk} and gp75 binding proteins under conditions known to alter gp75 expression in PC12 cells. PC12 cells were exposed to NGF for 0 to 7 days. Thereafter, [^{125}I]NGF was crosslinked to its receptors and subsequently immunoprecipitated by antibodies specific for the gp140^{trk} or gp75 binding proteins. The amount of gp140^{trk} detected is small relative to the amount of gp75 protein on the surface of naive PC12 cells. With NGF treatment, levels of gp140^{trk} on the cell surface increase while levels of gp75 decrease. These changes in NGF binding protein levels peak after 4 days of NGF treatment and correlate temporally with a rise in choline acetyltransferase activity (ChAcT). Thus the increases in gp140^{trk} and decreases in gp75 parallel the enhanced responsiveness to NGF induced by prior exposure to the growth factor. We are currently studying the regulation of message for each of these binding proteins and the total amount of gp140^{trk} and gp75 as a function of NGF treatment in PC12 cells.