Instituto Juan March
de Estudios e Investigaciones

14 CENTRO DE REUNIONES
INTERNACIONALES SOBRE BIOLOGÍA

Workshop on

Frontiers of Alzheimer Disease

Organized by

B. Frangione and J. Avila

J. Avila
K. Beyreuther
D. D. Cunningham
A. Delacourte
B. Frangione
C. Gajdusek
M. Goedert
Y. Ihara
K. Iqbal
K. S. Kosik
C. Milstein
D. L. Price
A. Probst
N. K. Robakis
A. D. Roses
S. S. Sisodia
C. J. Smith
W. G. Turnell
H. M. Wisniewski

Instituto Juan March
de Estudios e Investigaciones

CENTRO DE REUNIONES INTERNACIONALES
SOBRE BIOLOGÍA

14

Workshop on
Frontiers of Alzheimer Disease

Organized by

B. Frangione and J. Avila

J. Avila
K. Beyreuther
D. D. Cunningham
A. Delacourte
B. Frangione
C. Gajdusek
M. Goedert
Y. Ihara
K. Iqbal
K. S. Kosik
C. Milstein
D. L. Price
A. Probst
N. K. Robakis
A. D. Roses
S. S. Sisodia
C. J. Smith
W. G. Turnell
H. M. Wisniewski

The lectures summarized in this publication were presented by their authors at a workshop held on the 19th through the 21st of April, 1993, at the Instituto Juan March.

Depósito legal: M-18.012/1993
ISBN: 84-7919-513-4
Impresión: Ediciones Peninsular. Tomelloso, 27. 28026 Madrid

INDEX

PAGE

PROGRAMME..... 7

INTRODUCTION: J. Avila..... 9

CYTOSKELETAL PATHOLOGY OF AD..... 13

K. IQBAL: MECHANISMS OF ALZHEIMER NEUROFIBRILLARY DEGENERATION: ROLE OF ABNORMALLY PHOSPHORYLATED TAU... 15

J. AVILA: TAU PHOSPHORYLATION AND SELF-ASSOCIATION: IMPLICATIONS IN ALZHEIMER´S DISEASE..... 17

M. GOEDERT: TAU PROTEIN AND ALZHEIMER´S DISEASE..... 18

Y. IHARA: UBIQUITINATION OF THE TAU PAIRED HELICAL FILAMENTS (PHF)..... 20

A.DELACOURTE: ABNORMALLY PHOSPHORYLATED TAU PROTEINS: WHAT DO THEY TELL US ABOUT BRAIN AGING, ALZHEIMER´S DISEASE AND OTHER NEURODEGENERATIVE DISORDERS?..... 24

K.S. KOSIK: INTRANEURONAL COMPARTMENTS OF THE AMYLOID PRECURSOR PROTEIN..... 26

C.J. SMITH: STUDIES ON TAU PHOSPHORYLATION AND β-AMYLOID PRECURSOR PROTEIN (APP) EXPRESSION REVEAL PARALLELS BETWEEN THE PROCESSES OF NEURONAL DIFFERENTIATION AND DEGENERATION..... 27

AMYLOID IN AD: RECENT ADVANCES IN PATHOGENESIS BIOCHEMISTRY AND GENETICS..... 31

K.BEYREUTHER: ALZHEIMER βA4 AMYLOID (β PROTEIN) AND THE CAUSE OF ALZHEIMER´S DISEASE..... 33

B. FRANGIONE: ALZHEIMER´S DISEASE - DUTCH VARIANT.... 36

A.D. ROSES: GENETIC ASSOCIATION AND ISOFORM-SPECIFIC EFFECTS OF APOLIPOPROTEIN E IN LATE-ONSET FAMILIAL AND SPORADIC ALZHEIMER´S DISEASE..... 38

H.M. WISNIEWSKI: β-PROTEIN IMMUNOREACTIVITY, FIBRILLOGENESIS AND ITS IMPACT ON NEUROPIL AND VESSELS..... 40

S.S. SISODIA: STUDIES ON APP BIOLOGY; EXPRESSION AND CHARACTERIZATION OF A NOVEL APP HOMOLOG, APLP2, AND ANALYSIS OF TRANSGENIC MICE EXPRESSING THE ENTIRE HUMAN APP GENE..... 41

PAGE

N.K. ROBAKIS: PROCESSING AND BIOLOGICAL ACTIVITY OF APP..... 42

D.D. CUNNINGHAM: REGULATION OF NEURONS AND ASTROCYTES BY PROTEASE NEXIN-1 AND THROMBIN: POSSIBLE SIGNIFICANCE IN INJURY, INFLAMMATION AND ALHZEIMER´S DISEASE. 43

A. PROBST: THE EARLY SENILE PLAQUE: A REAPPRAISAL.... 45

W.G. TURNELL: BINDING OF THE DYE CONGO RED TO THE AMYLOID PROTEIN PIG INSULIN REVEALS A NOVEL HOMOLOGY AMONGST AMYLOID-FORMING PEPTIDE SEQUENCES..... 48

ANIMAL MODELS..... 49

D.L. PRICE: ANIMAL MODELS OF ALZHEIMER´S DISEASE..... 51

C. GAJDUSEK: NUCLEATION OF AMYLOIDOGENESIS IN INFECTIOUS AND NONINFECTIOUS AMYLOIDOSES OF BRAIN..... 56

POSTERS..... 63

K.C. BREEN: A DECREASE IN SERUM SIALYLTRANSFERASE LEVELS IN ALZHEIMER´S DESEASE..... 65

F. COLLIA: STUDY WITH PEROXIDASE (HRP) OF THE CONTRALATERAL PROJECTIONS OF THE NEOCORTEX IN AGED ALBINO RATS..... 66

F. CORIA: ENZYMATIC DIGESTION PATTERNS OF THE AMYLOID PROTEIN PRECURSOR IN THE CSF OF PATIENTS WITH ALZEIMER´S DISEASE..... 67

F. CORIA: LEVELS OF AMYLOID PROTEIN PRECUSOR IN THE CSF OF PATIENTS WITH ALZHEIMER´S DISEASE..... 68

A. CHAUHAN: AMYLOID BETA-PROTEIN STIMULATES CASEIN KINASE I AND CASEIN KINASE II ACTIVITIES..... 69

D.C. DAVIES: SYNAPSE LOSS FROM THE HIPPOCAMPUS IN ALZHEIMER´S DISEASE..... 70

N. MAHY: DIFFERENTIAL BRAIN AREA VULNERABILITY TO LONG TERM SUBCORTICAL EXCITOTOXIC LESIONS..... 71

F. MAYOR: ALZHEIMER β-AMYLOID PRECURSOR PROTEIN PROCESSING IN C6 GLIOMA CELLS..... 72

R. MENA: DIFFERENTIAL LABELLING OF A SUBPOPULATION OF PLAQUES WITH MAB 423 AND FLUORESCENT MARKER FOR INTRACELLULAR TANGLES..... 73

PAGE

E. MONTEJO DE GARCINI: TAU TRANSFECTION IN COS-1 CELLS STABILIZES CENTROSOME-INDEPENDENT MICROTUBULES AND PROMOTES THE EXTENSION OF CYTOPLASMIC PROCESSES... 74

F. MORA: ASTROCYTES, NORMAL AGING AND ALZHEIMER DISEASE..... 75

M. MORTILLA: LOOKING FOR A NOVEL LOCUS FOR FAMILIAL ALZHEIMER´S DISEASE..... 76

M. NOVAK: TRUNCATION OF TAU IN ALZHEIMER´S DISEASE... 77

R. OLIVA: APP VARIANTS IN SPANISH ALZHEIMER AND CONTROL CASES..... 79

J. SATRÚSTEGUI: CALCIUM REGULATION IN FIBROBLASTS DERIVED FROM SKIN BIOPSIES WITH VARYING AMYLOID β-PROTEIN DEPOSITION..... 80

C. VIGO-PELFREY: THE ROLE OF βAMYLOID PEPTIDE AS A DIAGNOSTIC AND PROGNOSTIC MARKER OF ALZHEIMER´S DISEASE..... 81

F. WANDOSELL: DIFFERENCES ON β-AMYLOID PRECURSOR PROTEIN GLYCOSYLATION AFTER BRAIN DAMAGE..... 82

SUMMARY: J. Avila..... 83

LIST OF INVITED SPEAKERS..... 87

LIST OF PARTICIPANTS 93

PROGRAMME

FRONTIERS OF ALZHEIMER DISEASE

MONDAY, April 19th, 1993

Introductory Remarks: D.L. Price.

Cytoskeletal Pathology of AD.
Chairperson: D.L. Price.

K. Iqbal - Mechanisms of Alzheimer Neurofibrillary Degeneration: Role of Abnormally Phosphorylated Tau.

J. Avila - Tau Phosphorylation and Self-Association: Implications in Alzheimer´s Disease.

M. Goedert - Tau Protein and Alzheimer´s Disease.

Y. Ihara - Ubiquitination of the Tau in Paired Helical Filaments (PHF).

Cytoskeletal Pathology of AD.
Chairperson: C. Milstein.

A.Delacourte - Abnormally Phosphorylated Tau Proteins: What Do They Tell Us About Brain Aging, Alzheimer´s Disease and Other Neurodegenerative Disorders?

K.S. Kosik - Intraneuronal Compartments of the Amyloid Precursor Protein.

C.J. Smith - Studies on Tau Phosphorylation and δ-Amyloid Precursor Protein (APP) Espression Reveal Parallels between the Processes of Neuronal Differentiation and Degeneration.

Poster Discussion:
- M. Novak.
- E. Montejo de Garcini.

C. Gajdusek - Cerebral Amyloidosis of Normal Aging, Alzeimer´s Disease, and Other Presenile Dementias. (Lecture Open to the Public).

TUESDAY, April 20th, 1993

Amyloid in AD: Recent Advances in Pathogenesis Biochemistry and Genetics.
Chairperson: D.D. Cunningham.

K.Beyreuther - Alzheimer βA4 Amyloid (β Protein) and the Cause of Alzheimer's Disease.

B. Frangione - Alzheimer's Disease - Dutch Variant.

A.D. Roses - Genetic Association and Isoform-specific Effects of Apolipoprotein E in Late-onset Familial and Sporadic Alzheimer's Disease.

H.M. Wisniewski - β-Protein Immunoreactivity, Fibrillogenesis and its Impact on Neuropil and Vessels.

S.S. Sisodia - Studies on APP Biology; Expression and Characterization of a Novel APP Homolog, APLP2, and Analysis of Transgenic Mice Expressing the Entire Human APP Gene.

N.K. Robakis - Processing and Biological Activity of APP.

D.D. Cunningham - Regulation of Neurons and Astrocytes by Protease Nexin-1 and Thrombin: Possible Significance in Injury, Inflammation and Alhzeimer's Disease.

A. Probst - The Early Senile Plaque: A Reappraisal.

W.G. Turnell - Binding of the Dye Congo Red to the Amyloid Protein Pig Insulin Reveals a Novel Homology amongst Amyloid-forming Peptide Sequences.

WEDNESDAY, April 21st, 1993

Animal Models.
Chairperson: N.K. Robakis.

D.L. Price - Animal Models of Alzheimer's Disease.

C. Gajdusek - Nucleation of Amyloidogenesis in Infectious and Noninfectious Amyloidoses of Brain.

Poster Discussion:
- F. Mayor.
- C. Vigo-Pelfrey.
- R. Oliva.

General discussion.

INTRODUCTION

J. Avila

Alzheimer's disease is an enourmous and increasing problem. It is the fourth leading cause of death in the industrialized nations. Recently, Dr. Price has indicated that 19% of Spanish people over 75 years may be afflicted with the disease, and in our country a total of 300000 persons may suffer it. An estimated 88 $ billion a year is spent in direct care of Alzheimer's disease patients in the U.S. and this does not begin to touch the cost to the families who still face minimal relief services, barriers to nursing home admission and the other severe problems associate with the disease.

To discuss about the current research, at a cellular and molecular level, on Alzheimer's disease, the present meeting was held in the Fundación Juan March. In the workshop about 40 scientists, including well-established leaders in the field (see list of participants) talk about their later findings.

Cytoskeletal Pathology of AD

MECHANISMS OF ALZHEIMER NEUROFIBRILLARY DEGENERATION: ROLE OF ABNORMALLY PHOSPHORYLATED TAU. Khalid Iqbal and Inge Grundke-Iqbal. Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314, USA.

Alzheimer disease is characterized clinically by dementia, and histopathologically by the presence of extensive neurofibrillary degeneration and brain ß-amyloidosis. The ß-amyloidosis alone, in the absence of Alzheimer neurofibrillary changes does not produce the disease clinically. Alzheimer neurofibrillary changes, which are mostly formed of paired helical filaments (PHF) and 2.1 nm tau filaments (1-2), occur as neurofibrillary tangles in the neuronal cell soma, neuropil threads in the dystrophic neurites in the neuropil, and as dystrophic neurites surrounding the extracellular ß-amyloid in the neuritic (senile) plaques. Microtubule associated protein tau is the major protein subunit of PHF (3-4). Tau protein in the affected neurons is hyperphosphorylated (5-7). It contains on average 5-10 PO_4 as compared to 2-3 PO_4 per normal tau molecule. To date nine abnormal phosphorylation sites on PHF-tau have been identified. Seven of these are canonical sites for proline-directed protein kinases, and two of the nine sites are phosphorylated by other protein kinases. The level of tau in AD neocortex is several fold higher than in aged control cases, but this increase is in the form of the abnormally phosphorylated protein (8). The microtubule associated proteins from AD brain do not promote the assembly of microtubules in vitro, whereas the in vitro dephosphorylated PHF polypeptides stimulate the assembly. The activities of phosphoseryl/phosphothreonyl protein phosphatase -1 and -2A and non-receptor phosphotyrosyl phosphatase are decreased in AD brain (9). It is suggested (i) that a defect(s) in the protein phosphorylation/dephosphorylation system leads to a hyperphosphorylation of tau, and (ii) that this abnormal phosphorylation and polymerization of tau into PHF most probably lead to a breakdown of the microtubule system and consequently to neuronal dysfunction and degeneration. (Supported in part by Alzheimer's Association Zenith Award; NIH grants AG 05892, AG 08076, AG 04220, NS 18105).

REFERENCES:

1. Ruben G.C., Iqbal K., Grundke-Iqbal I., Wisniewski H.M., Ciardelli T.L., and Johnson J.E. Jr. (1991) The microtubule-associated protein tau forms a triple-stranded left-hand helical polymer. *J. Biol. Chem.* **266,** 22019-22027.

2. Ruben G.C., Iqbal K., Wisniewski H.M., Johnson J.E. Jr., and Grundke-Iqbal I. (1992) Alzheimer neurofibrillary tangles contain 2.1 nm filaments structurally identical to the microtubule-associated protein tau: a high resolution transmission electron microscope study of tangles and senile plaque core amyloid. *Brain Res.* **590,** 164-179.

3. Iqbal K., Zaidi T., Thompson C.H., Merz P.A., and Wisniewski H.M. (1984) Alzheimer paired helical filaments: bulk isolation, solubility and protein composition. *Acta. Neuropathol. (Berl.)* **62,** 167-177.

4. Grundke-Iqbal I., Iqbal K., Quinlan M., Tung Y.-C., Zaidi M.S., and Wisniewski H.M. (1986) Microtubule-associated protein tau: a component of Alzheimer paired helical

filaments. *J. Biol. Chem.* **261,** 6084-6089.

5. Grundke-Iqbal I., Iqbal K., Tung Y.-C., Quinlan M., Wisniewski H.M., and Binder L.I. (1986) Abnormal phosphorylation of the microtubule-associated protein tau in Alzheimer cytoskeletal pathology. *Proc. Natl. Acad. Sci. USA* **83,** 4913-4917.

6. Iqbal K., Grundke-Iqbal I., Zaidi T., Merz P.A., Wen G.Y., Shaikh S.S., Wisniewski H.M., Alafuzoff I., and Winblad B. (1986) Defective brain microtubule assembly in Alzheimer's disease. *Lancet* **2,** 421-426.

7. Iqbal K., Grundke-Iqbal I., Smith A.J., George L., Tung Y.-C., and Zaidi T. (1989) Identification and localization of a tau peptide to paired helical filaments of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* **86,** 5646-5650.

8. Khatoon S., Grundke-Iqbal I., and Iqbal K. (1992) Brain levels of microtubule-associated protein tau are elevated in Alzheimer's disease: a radioimmuno-slot-blot assay for nanograms of the protein. *J. Neurochem.* **59,** 750-753.

9. Gong C.-X., Singh T.J., Grundke-Iqbal I., and Iqbal K. Phosphoprotein phosphatase activities in Alzheimer disease brain. *J. Neurochem.* In press.

Tau phosphorylation and self-association: implications in Alzheimer's disease

J. Avila, J. García de Ancos, M.D. Ledesma, J. Díaz-Nido and I. Correas

Centro de Biología Molecular (CSIC-UAM) Universidad Autónoma, Facultad de Ciencias, 28049-Cantoblanco, Madrid, Spain

In the neuron, the major role of tau protein may be the stabilization of microtubules, mainly within axons. This stabilization depends on the ability of tau to bind to tubulin and is thought to be regulated by phosphorylation. A reduced affinity of phosphorylated tau for tubulin may arise from induced conformational changes in tau molecule and/or direct phosphorylation on sites at its tubulin-binding domain. Tau binding to tubulin might be abolished upon hyperphosphorylation in neurons from patients with senile dementia of Alzheimer´s type (AD). Thus, there is a great interest in elucidating both the protein kinases implicated in tau hyperphosphorylation in AD and the steps needed for the formation of paired helical filaments (PHFs), aberrant structures present in the patient´s brains.

We have attempted to determine: 1) which protein kinase phosphorylates tau protein at the tubulin-binding domain to analyze whether or not a modification in that region results on a reduced interaction between tau and tubulin; 2) the self-association abilities of the different tau isoforms and the region of tau molecule involved in that interaction as a possible prior step for PHF formation; and 3) the isolation of a soluble precursor of the abnormally hyperphosphorylated tau which is present in PHFs (PHF-tau).

We have found that protein kinase C (PKC) is responsible for phosphorylating tau protein at its tubulin-binding domain. PKC phosphorylation at that tau region results on a reduced tau-tubulin interaction. Thus, phosphorylation of the tubulin-binding domain of tau protein by PKC may constitute a mechanism to modify the neuronal cytoskeleton in response to the neurotransmitters that activate PKC. The abnormal and uncontrolled activation of PKC may lead to hyperphosphorylation of tau with a subsequent destabilization of the microtubule cytoskeleton.

With regard to the tau self-association studies, we have determined that tau isoforms containing three tubulin-binding repeats form covalently-bound dimers more efficiently than tau isoforms containing four repeats. This dimer-forming ability is notably diminished in the presence of reducing agents, thus suggesting the involvement of cysteine residues. Additionally, tau forms larger aggregates which are observed even in the presence of reducing agents. These results support the idea that other regions in the tau molecule, besides the Cys-containing tubulin-binding region, also contribute to tau self-association. Tau dimerization and aggregation may be prior steps to PHF formation.

Finally, we have isolated a hyperphosphorylated tau protein fraction present in soluble form in AD brain cytosol, a possible precursor of PHF-tau, using iron-chelated affinity chromatography. This tau fraction is extensively phosphorylated at Ser/Thr-Pro sites, however, it remains soluble which suggests that other modifications are needed for PHF assembly.

All these data together suggest that hyperphosphorylation of tau at Ser/Thr-Pro sites by PDPKs probably is not the only modification of tau required for PHF formation.

Tau protein and Alzheimer's disease.

Michel Goedert
MRC Laboratory of Molecular Biology
Hills Road
Cambridge CB2 2QH, U.K.

Abundant senile plaques and neurofibrillary lesions constitute the major neuropathological characteristics of Alzheimer's disease. Neurofibrillary lesions appear within the vast majority of nerve cells that degenerate during the course of the disease, where their presence is indicative of dementia. Paired helical filaments (PHFs) are the principal fibrous structures of the neurofibrillary lesions (neurofibrillary tangles, neuropil threads and senile plaque neurites). Recent work has shown that PHFs consist predominantly and probably entirely of abnormally phosphorylated microtubule-associated protein tau. This is strongly supported by the demonstration that filaments like those found in Alzheimer's disease can be formed in vitro from recombinant tau protein fragments (1,2).

Tau is a group of developmentally regulated proteins generated from a single gene by alternative mRNA splicing that promote microtubule assembly and stabilisation. The most striking feature of the primary structure of tau is stretches of 31 or 32 amino acids, repeated three or four times in the carboxy-terminal half of the molecule, that constitute microtubule binding units. Additional isoforms contain 29 or 58 amino acid inserts in the amino-terminal region in conjunction with three or four repeats, giving rise in human brain to a total of six isoforms. The shortest human brain tau comprises 352 amino acids and contains three repeats, whereas the largest isoform is 441 amino acids in length and contains four repeats and the 58 amino acid insert (3). All isoforms are expressed in adult human brain, whereas only the shortest isoform is expressed in immature brain. Additional tau isoforms are found in the peripheral nervous system; they are characterised by the presence of a large insert in the amino-terminal half (4,5). Thus, in the rat, the largest brain tau isoform is 432 amino acids in length, whereas the largest peripheral nervous system isoform comprises 686 amino acids. Tau is a phosphoprotein, as evidenced by an increase in its gel mobility following dephosphorylation. This increase is more pronounced for immature than adult tau, indicating that immature tau is phosphorylated at different sites from adult tau.

In Alzheimer's disease tau is abnormally phosphorylated; it self-associates through the microtubule-binding domain to form the PHF. PHF tau is unable to bind to microtubules, but binds following dephosphorylation, indicating the deleterious functional consequences of abnormal phosphorylation. On SDS-polyacrylamide gels PHF tau runs as three bands of 60, 64 and 68 kDa apparent molecular mass that do not align with native or recombinant tau. Alignment is achieved following alkaline phosphatase treatment at high temperature (6). The relative intensities of the six bands are similar to those found for soluble tau extracted from normal adult brain, indicating that PHFs contain all six isoforms in proportion to their natural abundance, but in an abnormally phosphorylated state.

Antibodies have been produced that recognise PHF tau in a phosphorylation-dependent manner and fail to recognise normal adult tau, permitting to identify some of the abnormally phosphorylated sites. Thus, antiserum T3P recognises S396 in a phosphorylated state (7); antibody AT8 recognises phosphoserine202 (8,9), while antibody AT18 requires T231 and/or S235 to be phosphorylated. Surprisingly, these

antibodies also recognise immature human tau. The abnormal phosphorylation sites identified by T3P, AT8 and AT18 are all SP or TP sites. The largest human brain tau isoform contains 17 such sites; they flank the microtubule-binding repeat region and the cluster just amino-terminal to the repeat region overlaps a strongly basic region of the protein.

Protein kinases and/or protein phosphatases with a specificity for SP and TP sites are probably instrumental in the abnormal phosphorylation of tau. Recently, p42 mitogen-activated protein (MAP) kinase was shown to phosphorylate recombinant tau at a number of sites, including S202 and S396 (10,11). Of the major brain protein phosphatases tau phosphorylated in this manner could only be dephosphorylated by protein phosphatase 2A, with phosphatase $2A_1$ as the most effective form of the enzyme (11). Future experiments will show whether p42 MAP kinase and other protein kinases with a similar specificity are more active in fetal and Alzheimer brains than in normal adult brain or whether protein phosphatase $2A_1$ is less active. The developing brain may constitute a useful system for studying the regulation of tau phosphorylation. Elucidation of the mechanisms underlying these phenomena could lead to the development of strategies to prevent or retard the neurofibrillary pathology of Alzheimer's disease.

(1) Wille H, Drewes G, Biernat J, Mandelkow EM & Mandelkow E (1992) J. Cell Biol. 118: 573-584.
(2) Crowther RA, Olesen OF, Jakes R & Goedert M (1992) FEBS Lett. 309: 199-202.
(3) Goedert M, Spillantini MG, Jakes R, Rutherford D & Crowther RA (1989) Neuron 3: 519-526.
(4) Goedert M, Spillantini MG & Crowther RA (1992) Proc. Natl. Acad. Sci. USA 89: 1983-1987.
(5) Couchie D, Mavilia C, Georgieff IS, Liem RKH, Shelanski ML & Nunez J (1992) Proc. Natl. Acad. Sci. USA 89: 4378-4381.
(6) Goedert M, Spillantini MG, Cairns NJ & Crowther RA (1992) Neuron 8: 159-168.
(7) Lee VM-Y, Balin BJ, Otvos L & Trojanowski JQ (1991) Science 251: 675-678.
(8) Biernat J, Mandelkow EM, Schröter C, Lichtenberg-Kraag B, Steiner B, Berling B, Meyer H, Mercken M, Vandermeeren A, Goedert M & Mandelkow E (1992) EMBO J. 11: 1593-1597.
(9) Goedert M, Jakes R, Crowther RA, Six J, Lübke U, Vandermeeren M, Cras P, Trojanowski JQ & Lee VM-Y (1993) Proc. Natl. Acad. Sci. USA, in the press.
(10) Drewes G, Lichtenberg-Kraag B, Döring F, Mandelkow EM, Biernat J, Dorée M & Mandelkow E (1992) EMBO J.11: 2131-2138.
(11) Goedert M, Cohen ES, Jakes R & Cohen P (1992) FEBS Lett 312: 95-99.

Ubiquitination of the tau in Paired Helical Filaments (PHF)

Yasuo Ihara

Selective intracellular protein degradation is essential for modulation of the levels of short-lived regulatory proteins and removal of abnormal or damaged proteins. Ubiquitin, a 76 amino acid polypeptide, marks a target protein through an isopeptide bond and its ubiquitinated form is efficiently degraded by a 26S protease complex. Ubiquitination of protein occurs as a monoubiquitinated form or a multiubiquitin chain: the former may not be a degradation signal, but may act as a modulator of target protein's function, while the latter, Lys-48-linked ubiquitin-ubiquitin conjugate, works as a strong degradation signal, when joined to Lys in a target protein.

In Alzheimer's disease (AD), paired helical filaments (PHF), which are a fibril composed of two 10 nm filaments wound around each other, accumulate progressively in neuronal cell bodies as neurofibrillary tangles and in their fine processes as curly fibers or neuropil threads. We previously showed that ubiquitin is a component of PHF and concluded that tangle-bearing neurons respond to PHF, a seemingly inert structure, by actively ubiquitinating them. To date ubiquitin-immunoreactivities have been demonstrated in various cytoplasmic inclusion bodies in degenerative diseases, which include Pick bodies in Pick's disease, Lewy bodies in Parkinson's disease and diffuse Lewy body disease, Lewy-like bodies in amyotrophic lateral sclerosis, and Mallory bodies in alcoholic liver disease. In addition, ubiquitin-immunoreactive aggregates have recently been shown to emerge even in normal human brain in an age-related fashion. However, the significance of ubiquitin in these inclusions remains largely unknown. We do not know exactly whether ubiquitin in the aggregates exist in a free form or a conjugated form and whether or not ubiquitin in them works as a degradation signal for the ubiquitin pathway.

To make this clear, we attempted to identify the ubiquitin-targeted

protein in PHF. Immunoblot analysis with various tau antibodies of Sarkosyl-insoluble pellets prepared from AD brain showed diffuse smearing substances on SDSPAGE in addition to PHF-tau (A68), full-length tau with unusually slow mobilities. This smear pattern is characteristic of the immunoblot of the PHF-enriched fraction with the antibodies to the carboxyl-terminal portion of tau. On the other hand, DF2, a PHF monoclonal which was shown to specifically react with ubiquitin, did not label any distinct band, but rather labeled the whole smear in the blot. This result suggests that ubiquitin is not bound to PHF-tau but to the more processed and modified tau that shows a smear.

We then purified and characterized the smear, which is referred to as PHF-smear. Detailed peptide maps of PHF-smear, PHF-tau, and ubiquitin were made using *Achromobacter lyticus protease* I (API), a protease highly specific for Lys-X, and compared with each another. The profile of PHF-smear was very similar to the PHF-tau and ubiquitin profiles put together. However, U8, the most carboxyl-terminal API peptide from ubiquitin (see Figure 1), was undetectable in PHF-smear. This suggests that U8 in PHF-smear is ligated to a certain peptide through an isopeptide bond and elutes elsewhere. Several small peaks were found to be characteristic of PHF-smear and subjected to amino acid sequence and mass spectrometric analyses.

Sequence analysis of the largest peak among those gave the two ubiquitin sequences, EGIPPD $\cdots\cdot$ (U6) and ESTLHL $\cdots\cdot$ (U8), with almost equal yields. Lys-48 in U6 was hardly recovered and the sequence continued to QLEDG $\cdots\cdot$ (U7). This strongly suggests that the ε-amino group of Lys-48 is modified because API is unable to cleave off at the carboxyl side of Lys-48. Since a multiubiquitin chain, when digested with API, releases a branched peptide, U8-ligated U6-U7 at Lys-48, the above observation indicates that this particular peak contains the Y-shaped API peptide derived from a multiubiquitin chain.

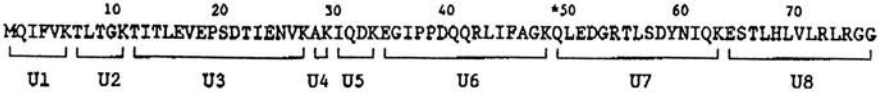
The analysis of the other peaks characteristic of PHF-smear showed that

each contained two amino-terminal sequences: one is ESTLHLVLR ···· (U8) and the other is a tau-derived sequence, SRLQT ···· (241-257), NVKSK (255-259), HVPGGG ···· (299-; a peptide specific for four-repeat tau), VQIVYKPVD ···· (306-; a peptide specific for three-repeat tau) (the numbering used here is according to the 441-amino acid-human tau isoform). Overall, ubiquitin is conjugated with the tau in PHF at Lys-254, 257, 311, and 317. Thus, the ubiquitin-targeted protein in PHF is tau and the multiubiquitin is linked to tau, although its proportion in ubiquitin in PHF is very small (~20%). Most interestingly, these ubiquitin-conjugated lysine residues are all localized in the microtubule-binding region of tau, especially on the amino-terminal sides of the first and third microtubule-binding domains, while abnormal phosphorylation sites surround the binding region.

A rough estimation based on the HPLC profiles suggests that the molar ratios of tau and ubiquitin are almost equal. This again confirms that tau and ubiquitin are the major components of a class of PHF that shows a smear in the blot (PHF-smear); ubiquitin is a major component of processed PHF.

We do not know why the multiubiquitin chain comprizes a very small proportion of ubiquitin in PHF. Multiubiquitinated tau in PHF may be rapidly degraded so that only a very small amount of the degradation intermediate can be detected in the steady state. Alternatively, the ubiquitin pathway in tangle-bearing neurons may have some inherent defects in multiubiquitination. When compared to the multiubiquitin chain, the monoubiquitinated form may not work as a strong degradation signal. This may partly explains progressive accumulation of PHF despite ubiquitination in subsets of neurons.

Figure 1



ABNORMALLY PHOSPHORYLATED TAU PROTEINS : WHAT DO THEY TELL US ABOUT BRAIN AGING, ALZHEIMER'S DISEASE AND OTHER NEURODEGENERATIVE DISORDERS ?

DELACOURTE ANDRÉ AND PATRICK VERMERSCH.
U. 156 INSERM, Place de Verdun 59045 Lille France

Alzheimer's disease (AD) results from two main pathological events: amyloid deposition and neurofibrillary degeneration (NFD). The understanding of the biochemical dysfunction leading to NFD and neuronal cell death strongly relies upon the discovery of reliable markers of the disease. **Genetic markers** have already been discovered, showing for example that a mutation on Amyloid Protein Precursor (APP) is sufficient to lead to the disease. Others, on chromosomes 19 and 14 are on the verge to be characterised. However, regarding the sporadic cases, which apparently represent most of AD cases, **biochemical markers** must be found to elucidate the aetiology of AD and the etiopathogenesis of the lesions, to set up an early diagnostic test and experimental models of the disease. In other words, an early and reliable biochemical marker of AD could lead to a rational and efficient therapeutic approach. In 1989 and 1990 (1, 2), we characterised a biochemical marker of NFD, namely Tau 64, 69, which are abnormally phosphorylated. This pathological Tau proteins are identical to A68 described by P. Davies (3) and also named Tau-PHF.

An immunoblot approach with polyclonal and monoclonal antibodies against Tau-PHF was used to quantify pathological Tau in the different Brodmann areas from Alzheimer patients (4). These studies confirm that the disease spreads like a chain reaction along cortico-cortical connections, from the entorhinal and hippocampal region to the associative neocortical areas and reaches the entire cortex and some subcortical nuclei (striatum for example) at the end-stage of the disease. The quantity of Tau-PHF in the isocortex was perfectly well correlated with the number of neurofibrillary tangles and dementia (5).

An analysis of non-demented elderly Controls with a Mini Mental State equal or superior to 28 revealed that abnormal Tau proteins are frequently found in the entorhinal cortex of controls aged above 70 and in the hippocampus of controls aged over 80. The isocortex was always spared in non-demented patients. In good agreement with other studies (6), our results show that entorhinal cortex and hippocampus are vulnerable during aging and are likely the first brain regions to degenerate during AD (7).

Tau 55, 64 and 69 were also found in the brain from patients with Down Syndrome, demonstrating that the biochemical dysfunctions in DS are of the "Alzheimer type" (8).

Even more remarkable is the detection of the abnormal Tau triplet in the prefrontal area 9 in most of the demented Parkinson Disease (PD) patients, sometimes in large amounts, while the detection in the occipital and cingular cortex was weak or absent. This striking pattern is unusual in AD and gives a biochemical evidence for AD changes in the cortex of demented PD patients; it also suggests that lesions of the prefrontal cortex may significantly contribute to the occurence of cognitive changes at least in some PD patients (9).

Progressive Supranuclear Palsy (PSP) is a neurological disorder with subcortical and cortical tangles labelled with anti-Tau. However, the biochemical profile of Tau proteins detected in PSP brain homogenates was significantly different: Tau 55 was never detected, and Tau 64 and 69 were slightly modified, with a different isoelectric point (10).
Together, using the biochemical characterisation of pathological Tau proteins, we were able to distinguish three types of degenerating process: 1) without a Tau pathology (Huntington Chorea, SLA) ; with a Tau pathology : 2) the Alzheimer type and 3) the PSP type.

The specificity of the pathological Tau triplet for Alzheimer pathology has already been used with success for a biochemical diagnosis performed on biopsies of young patients with atypical dementia. This specificity is also currently investigated in order to set up an early and peripheral diagnostic test on CSF.

Results accumulated on pathological Tau show that their expression in the isocortex is strongly associated with clinical symptoms of dementia. Further studies on animal or cell models expressing pathological Tau will likely open new therapeutical avenues.

REFERENCES: (1) Flament et al. **FEBS Lett.;** 1989, 247: 213-216 ; (2) Delacourte et al. **Acta Neuropathol.;** 1990, 80: 111-117 ; (3) Flament and Delacourte **Nature;** 1990, 346: 22 (4) Flament et al, **Acta Neuropathol.**, 1990, 80:212-215; (5) Vermersch et al **Acta Neuropathol.;** 1992, 85:48-54 ; (6)Braak and Braak, **Acta Neuropathol,** 1991, 82:239-259 ; (7) Vermersch et al **Neurosci. Lett.;** 1992, 144: 143-146., (8) Flament et al **Brain Res.;** 1990, 516: 15-19 ; (9) Vermersch et al, **Ann. Neurol;** 1993 in press ; (10) Flament et al **Acta Neuropathol.;** 1991, 81: 591-596.

INTRANEURONAL COMPARTMENTS OF THE AMYLOID PRECURSOR PROTEIN

Adriana Ferreira, Alfredo Caceres, Kenneth S. Kosik

Harvard Medical School and Center for Neurologic Diseases,

Department of Medicine (Division of Neurology),

Brigham and Women's Hospital, Boston, MA 02115

The amyloid precursor protein (APP) is the parent molecule from which β-amyloid protein is cleaved and deposits as amyloid fibrils in the senile plaques of Alzheimer's disease. In growing hippocampal neurons APP-immunoreactivity appears at the most distal limiting membrane of the growth cone. By 15 days in culture, when neurites have already attained dendritic and axonal identities, APP-immunoreactivity is observed predominantly in a subset of axons. Two patterns of immunoreactivity are observed within axons: difuse staining and punctate staining. The punctate APP-labeling co-localizes with clathrin-immunoreactive clusters of vesicular-like structures.
Following cytosolic acidification, late endosomes as labeled with lucifer yellow get redistributed from the cell body to the neuronal processes (1). Acidification of the media resulted in the translocation of APP-immunoreactivity to all axons where it co-localized with late endosomes (2).

In neurons APP moves anterograde in the fast phase of axonal transport (3). Translocation of APP-immunoreactivity to the neurites of hippocampal cultures can be inhibited by anti-sense oligonucleotides to the kinesin heavy chain (2,4). This finding suggests that the anterograde transport of APP is mediated by the plus end-directed microtubule-based motor, kinesin.

While important missing steps in understanding the formation of amyloid fibrils remain, the trace of the disease process is completely lost in the transition to the neuritic lesions, including the neurofibrillary tangles and the dystrophic neurites. Observed within affected neurons is the dissolution of various cytoskeletal elements, particularly the microtubules
and the polymerization of the microtubule-associated protein, tau, into filaments. This event appears to be mediated by the excessive phosphorylation of tau protein. The direction of future studies must learn the nature of the insult which activates the responsible kinases and the relationship of this trigger to APP.

References:

1. Parton RG, Dotti CG, Bacallao R, Kurtz I, Simons K, Prydz K (1991). pH-induced microtubule-dependent redistribution of late endosomes in neuronal and epithelial cells. J Cell Biol. 113:261-274.

2. Koo EH, Sisodia SS, Archer DR, Martin LJ, Weidemann A, Beyreuther K, Fischer P, Masters CL, Price DL (1990). Precursor of amyloid protein in Alzheimer disease undergoes fast anterograde axonal transport. Proc Natl Acad Sci USA 87:1561-1565.

3. Ferreira A, Niclas J, Vale RD, Banker G, Kosik KS. (1992). Suppression of kinesin expression in cultured hippocampal neurons using antisense oligonucleotides. J Cell Biol. 117:595-606.

4. Ferreira A, Caceres A, Kosik KS. Intraneuronal compartments of the amyloid precursor protein. J. Neurosci. 1973; in press.

Studies on tau phosphorylation and β-amyloid precursor protein (APP) expression reveal parallels between the processes of neuronal differentiation and degeneration.

Carthage J. Smith[1], Jean-Pierre Brion[2], Philippe Brachet[3] and Brian H. Anderton[1].

Department of Neuroscience, Institute of Psychiatry, London, SE5 8AF. [2]Laboratory of Pathology, Université Libre de Bruxelles, 1070 - Brussels, Belgium. [3]INSERM U298, CHRU, Angers, France.

The clearest neuropathological correlate of Alzheimer's disease dementia is the presence of intra-neuronal neurofibrillary tangles, which at the electron microscope level are made up of paired helical filaments (PHF). These PHF are known to consist largely if not exclusively of abnormally hyperphosphorylated microtubule associated protein tau. A number of antibodies have now been characterised which recognise defined phosphorylated epitopes in PHF tau and we have used a panel of such antibodies to study the phosphorylation state of tau during rat brain development (1). Whereas 6 molecular isoforms of tau are present in adult human brain (2), we have shown, using a sensitive RT-PCR assay, that only the shortest tau isoform is expressed in rat brain up to postnatal day 3. However, size fractionation of heat soluble brain proteins on SDS-PAGE and subsequent Western analysis revealed two juvenile tau species migrating as a doublet of about 48KD. The slower migrating species was exclusively recognised by a panel of PHF-tau specific antibodies, including AT8, 8D8, RT97, SMI31 and SMI34 (3,4,5). Furthermore this immunoreactivity was phosphorylation dependent and could be abolished or reduced by alkaline phosphatase treatment. In contrast, the monoclonal antibody tau-1 (6), which requires a dephosphorylated epitope for reactivity, only recognised the slow migrating foetal tau species following prior dephosphorylation. A very similar pattern of tau immunoreactivity was subsequently confirmed in foetal human brain (at 28 weeks). These observations suggests that the kinase/phosphatase activities responsible for generating PHF-tau epitopes are active in the developing brain and possibly re-activated in neurodegenerative disease.

The second neuropathological hallmark characteristic of Alzheimer's disease are the senile plaques, which are a consequence of the extracellular deposition and aggregation of the 40-43 amino acid β/A4 peptide. This peptide is, in turn, generated from the β-amyloid precursor protein (APP), which exists as several alternatively spliced molecular isoforms. In neurones the major APP isoform contains 695 amino acids (APP_{695}) and one also finds longer isoforms (APP_{751} and APP_{770}) which are functionally distinguished by the insertion of a kunitz protease inhibitor domain. We have previously shown that neuronal differentiation of PC12 cells is associated with an increase in the $APP_{695}/APP_{751/770}$ ratio (7) and there is evidence that altered neuronal expression of APP isoforms, opposite to that seen during differentiation, may have a role in β/A4 deposition in Alzheimer's disease (8).

In order to investigate the relationship between expression of APP isoforms and neuronal degeneration, we chose the rat superior cervical ganglia (SCG) as an in vivo experimental model (9). In the neonate these sympathetic ganglia are nerve growth factor (NGF) dependent and in vivo administration of anti-NGF antiserum results in exaggerated neuronal degeneration (10). Sensitive S1-nuclease analysis of APP mRNA transcripts in the SCG, following NGF deprivation, revealed a coincident decrease in APP_{695} and augmentation of $APP_{751/770}$. these changes were specific to the SCG and were not seen in sensory ganglia. Subsequent in vitro studies, using primary dissociated cultures of sympathetic or cortical neurones, confirmed these changes in APP expression during neuronal degeneration.

Whilst the temporal sequence of events linking neuronal degeneration, altered APP expression and β/A4 deposition remains elusive, our results confirm that altered APP expression can occur as a result of neuronal insult (this effect being compounded where there is concurrent glial cell proliferation) and may be an important factor in β/A4 deposition.

References

1 Brion, J-P, Smith, C, Couck, A-M, Gallo, J-M and Anderton, BH (1993) Developmental changes in tau phosphorylation: fetal tau is transiently phosphorylated in a manner similar to paired helical filament tau characteristic of Alzheimer's disease. J. Neurochem., in press.

2 Goedert, M, Spillantini, MG, Potier, MC, Ulrich, J and Crowther, RA (1989) Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats; differential expression of tau protein mRNAs in human brain. EMBO J. 8, 393-399.

3 Mercken, M, Vandermeeren, M, Lubke U, Six J, Boons J, Van De Voorde A, Martin JJ and Gheuens J (1992) Monoclonal antibodies with selective specificity for Alzheimer tau are directed against phosphatase-sensitive epitopes. Acta Neuropathol. (Berl.) 84, 265-272.

4 Lichtenberg-Kraag B, Mandelkow E-M, Biernat J, Steiner B, Schroter C, Gustke N, Meyer HE and Mandelkow E (1992) Phosphorylation-dependent epitopes of neurofilament antibodies on tau protein and relationship with Alzheimer tau. PNAS 89, 5384-5388.

5 Brion J-P, Couck A-M, Robertson J, Loviny TLF and Anderton BH (1993) Neurofilament monoclonal antibodies RT97 and 8D8 recognise different modified epitopes in paired helical filament-τ in Alzheimer's disease. J. Neurochem. 60, 1372-1382.

6 Binder LI, Frankfurter A and Rebhun I (1985) The distribution of tau in the mammalian central nervous system. J. Cell Biol. 101, 1371-1378.

7 Smith CJ, Wion D and Brachet P (1991) Nerve growth factor induced neuronal differentiation is accompanied by differential splicing of β-amyloid precursor mRNAs in the PC12 cell line. Mol. Brain Res. 10, 351-354.

8 Johnson SA, McNeill T, Cordell B and Finch CE (1990) Relation of neuronal APP-751/APP-695 mRNA ratio and neuritic plaque density in Alzheimer's disease. Science 248, 854-857.

9 Smith CJ, Johnson Jr EM, Osborne P, Freeman RS, Neveu I and Brachet P (1993) NGF deprivation and neuronal degeneration trigger altered β-amyloid precursor protein gene expression in the rat superior cervical ganglia in vivo and in vitro.

10 Martin DP, Schmidt RE, DiStefano PS, Lowry OH, Carter JG and Johnson EM (1988) Inhibitors of protein synthesis and RNA synthesis prevent neuronal death caused by nerve growth factor deprivation. J. Cell Biol. 106, 829-844.

Amyloid in AD: Recent Advances in Pathogenesis Biochemistry and Genetics

ALZHEIMER βA4 AMYLOID (β PROTEIN) AND THE CAUSE OF ALZHEIMER'S DISEASE

Konrad Beyreuther1 and Colin L. Masters2, 1Center for Molecular Biology Heidelberg, University Heidelberg, Germany; 2Department of Pathology, University of Melbourne, Parkville, Victoria, Australia

The clinical phenotype of Alzheimer's disease (AD) is understood as retrograde development of higher order brain functions with synaptic and neuronal dysfunction and lately synaptic and neuronal loss. The genotype of AD is defined by amyloid deposition at synapses, in the media of brain vessels and in neurons. We identified and studied the amyloid βA4 protein which is the molecular hallmark of AD, and its precursor, a family of alternatively spliced transmembrane glycoproteins (APP) encoded by the APP gene on chromosome 21. APP is ubiquitously expressed by all cells which actively participate in cell-cell interaction. Cells that are capable to differentiate from non-adhesive to adhesive states such as leukocytes express APP at the protein level at the same time when they gain the function to interact with other cells and the extracellular matrix. Thus only activated T-cells and microglia show a correlation between APP synthesis and secretion and adhesivness. This is in accordance with the presence of APP binding sites for cell surface receptors such as heparan sulfate proteoglycans and molecules of the extracellular matrix. We were also able to show that the binding of APP to heparin-like molecules is modulated by zinc ions. This modulation is mediated through a novel zinc binding motif which is evolutionary highly conserved among the members of the APP superfamily. Therefore we propose a general role of APP's in the regulation of cell-cell contacts. In neurons APP serves a function at synaptic sites to where its is axonally transported, and at postsynaptic sites where it has been shown to be present in small vesicles.

The amyloid βA4 protein is derived from APP by proteolysis. In families with AD, mutations in and adjacent to the βA4 region have been linked with the disease providing compelling evidence for the central role that APP and the pathologic aggregated form of its breakdown product βA4 must play in brain function and dysfunction. At physiological concentrations (nanomolar) βA4 aggregation requires amino acid oxidation and protein cross-linking by radical generation systems. The addition of radical scavangers such as ascorbic acid, tocopherol derivatives, and free amino acids prevent radical induced βA4 aggregation.

The prevalence of βA4 deposition increases as a function of age in the normal population and in Down syndrome (DS). Our population surveys show that it takes 30 years for AD to develop in both, normal karyotypes and in individuals with DS. Thus, βA4 aggregates are a biologicial marker of AD, and its presence defines individuals at risk.

In human brain and muscle, APP, due to its preferential localization at pre- and post-synaptic sites, must serve a function in plasticity. In the light of our recent finding that free radicals are necessary to initiate the aggregation of βA4 molecules, it is suggested

that activation of microglial cells during neuro-degeneration causes radical damage of neuronal membranes thus enabling processing of APP to ßA4 and initiating ßA4 aggregation and depositioning. This results in amyloid formation at synapses, and thus in synaptic dysfunction, and lately in synaptic loss, and finally, after 30 years, in the clinical symptoms for AD.

REFERENCES

Bush AI, Williamson TG, L QX, Small DH, Moir RD, Currie J, Rumble B, Mönning U, Beyreuther K, Masters CL (1992) An Abnormality of Plasma Amyloid Protein Precursor in Alzheimer's Disease. *Ann Neurol* 32: 57-65

Dyrks T, Dyrks E, Hartmann T, Masters CL, Beyreuther K (1992) Amyloidogenicity of ßA4 and ßA4-bearing APP Fragments by Metal Catalysed Oxidation. J *Biol Chem* 267:18210-18217

Hilbich C, Kisters-Woike B, Reed J, Masters CL, Beyreuther K (1991) Aggregation and Secondary Structure of Synthetic Amyloid ßA4 Peptides of Alzheimer´s Disease. *J Mol Biol* **218**:149-163

Hilbich C, Kisters-Woike B, Reed J, Masters CL, Beyreuther K (1992) Substitutions of Hydrophobic Amino Acid Reduce the Amyloidogenicity of Alzheimer's Disease ßA4 Peptides *J Mol Biol* **228**: 460-473

Kang J, Lemaire H-G, Unterbeck A, Salbaum, JM, Masters CL, Grzeschik KH, Multhaup G, Beyreuther K, Müller-Hill B (1987)The precursor of Alzheimer's disease amyloid A4 protein resembles a cell surface receptor. *Nature* **325**:733-736

König G, Mönning U, Prior R, Banati R, Schreiter-Gasser U, Bauer J, Masters CL, Beyreuther K (1992) Identification of a Novel, Leukocyte-derived Isoform of the Alzheimer Amyloid Precursor Protein. *J Biol Chem* 267:1084-10809

Lemaire H-G, Salbaum JM, Multhaup G, Kang J, Bayney RM, Unterbeck A, K. Beyreuther K, Müller-Hill B (1989) The PreA4$_{695}$ precursor protein of Alzheimer's disease A4 amyloid is encoded by 16 exons. *Nucl Acid Res* **17**:517-522

Milward EA, Papadopoulos R, Fuller SJ, Moir RD, Beyreuther K, Masters CL (1992) The Amyloid Protein Precursor of Alzheimer's Disease Is A Mediator of the Effects of Nerve Growth Factor on Neurite Outgrouth. *Neuron* 9:129-137

Mönning U, König G, Banati RB, Mechler H, Czech C, Gehrmann J, Schreiter-Gasser U, Masters CL, Beyreuther K (1992) Alzheimer ßA4 Amyloid Precursor Proteins in Immuno-Competent Cellss. *J Biol Chem* **267**:23950-23956

Multhaup G, Bush A, Pollwein P, Masters C L, Beyreuther K (1992). Specific binding of the Alzheimer ßA4 precursor to collagen, laminin and heparin. *J. Protein Chem.* 11, 398-399.

Rumble B, Retallak R, Hilbich C, Simms G, Multhaup G, Martins R, Hockey A, Montgomery P, Beyreuther K, Masters CL (1989) Amyloid A4 protein and its precursor in Down´s syndrome and Alzheimer´s disease. *N Engl J Med* **320**:1446-1452

Schubert W, Prior R, Weidemann A, Dircksen H, Multhaup G, Masters CL, Beyreuther K (1991) Localization of Alzheimer ßA4 amyloid precursor protein at central and peripheral synaptic sites. *Brain Res* 663:184-194

Weidemann A, König G, Bunke D, Fischer P, Salbaum JM, Masters CL, Beyreuther K (1989): Identification, biogenesis and localization of precursors of Alzheimer's disease A4 amyloid protein. *Cell* **57**: 115-126

ALZHEIMER'S DISEASE - DUTCH VARIANT

Blas Frangione, M.D., Ph.D.
New York University Medical Center

The rare autosomal dominant form of cerebrovascular amyloidosis in Dutch patients, designated Hereditary Cerebral Hemorrhage with Amyloidosis - Dutch type (HCHWA-D), presents clinically between the ages of 45 and 65 with a stroke. Neuropathologically there is extensive deposition of amyloid in the vessel wall, preamyloid deposits, and early plaques. Neuritic plaques and neurofibrillary tangles are absent. The amyloid fibril is mainly composed of a 39 amino acid peptide that is similar to the amyloid β-protein (Aβ) extracted from AD brains. Recently it has been shown that Aβ is a normal degradation product of β precursor protein (βPP) and is found in biological fluids and cell supernatants.

A mutation was found in the APP gene from patients with HCHWA-D at nucleotide 1852 in exon 15 (APP_{695} numbering), where G is replaced by C. The latter causes a single amino acid substitution of Gln instead of Glu acid at codon 618 of APP_{695} (or 693 of APP_{770}) and corresponds to residue 22 of the Aβ. The significance of this mutation is corroborated by close linkage, with a Lod score of 7.59, between the APP gene and the disease. Furthermore this mutation has been found in all patients affected by Dutch amyloidosis tested so far.

The Dutch mutation accelerates fibril formation *in vitro*, correlating with amyloid deposition early in life. An important observation is that amyloid fibrils deposited in cerebral vessel walls of HCHWA-D contain both mutated and non-mutated Aβ in an equal ratio. A possible explanation is that mutation containing fibrils can act as an instructor, or template, facilitating the conversion of the amyloidogenic normal precursor to amyloid.

Several proteins also accompany amyloid fibril formation (e.g. P component, glycosaminoglycans, apolipoprotein E, etc.). These amyloid associated proteins may reduce solubility and stabilize the β-pleated structure in amyloid fibrils or their precursor proteins. These proteins may therefore be functioning as "pathological chaperones." We define "pathological chaperones" as a group of unrelated proteins that mediate β-pleated amyloid formation of polypeptide fragments, but are most likely not themselves components of the final fibrils. Pathological chaperones may function by binding specifically to amyloidogenic proteins that are found during aberrant protein degradation, thus preventing further proteolysis. Understanding how Aβ, normally a soluble peptide, is transformed into fibrils is a critical part of understanding the mechanism of neuronal dysfunction in AD.

GENETIC ASSOCIATION AND ISOFORM-SPECIFIC EFFECTS OF APOLIPOPROTEIN E IN LATE-ONSET FAMILIAL AND SPORADIC ALZHEIMER´S DISEASE

Allen D. Rosse[1], Ann M. Saunders[1], Donald E. Schmechel[1], Margaret A. Pericak-Vance[1], Peter St George-Hyslop[2] , Dmitry Goldgaber [3], Warren J. Strittmatter[1]

[1]Joseph and Kathleen Bryan Alzheimer's Disease Center, Division of Neurology, Duke University Medical Center, Durham, NC 27710; [2] Tanz Center for Neurogenetic Diseases, Toronto, Canada; [3] Dept. of Psychiatry, State University of New York, Stoney Brook, NY.

Alzheimer's disease is the leading cause of dementia in the elderly. Most patients do not have an obvious family history and are classified as sporadic. Genetic factors in early- and late-onset familial Alzheimer's disease (FAD) are now well documented. There are three defined loci for the comparatively rare autosomal dominant early-onset familial Alzheimer's Disease: 1) several mutations of the amyloid precursor protein on chromosome 21; 2) robust linkage of most of the remaining early-onset FAD families to CH 14q; and 3) an unknown locus excluded from CHs 21, 14 and 19 for a group of Volga-German families.

We reported linkage of late-onset FAD to the proximal long arm of CH 19 [1]. Several independent lines of evidence have led us to examine apolipoprotein E (apoE, protein; APOE, gene) in late-onset FAD as a candidate genetic factor. We observed several proteins in cerebrospinal fluid that bound to immobilized amyloid ß-peptide (ßA4) with high affinity, one of which was apoE. APOE was known to be coded within the region of CH 19 defined by linkage to late-onset FAD. Furthermore, antisera to ApoE stained senile plaques, neurofibrillary tangles, and cerebral vessel amyloid deposits in AD brains. We initially found that APOE, type ε4 allele (APOE4) was highly significantly associated with AD in late-onset families [2].

To test the hypotheses that: 1) late-onset FAD behaves as a complex genetic disease [3], we extended the analyses of APOE alleles to two clinical series of sporadic AD patients. In a prospective clinical series of patients diagnosed as probable sporadic AD (and spouse controls) presenting to the Memory Disorders Clinic, APOE4 was highly significantly associated with AD [0.36±0.042, AD versus 0.16±0.027, controls (allele frequency estimate±standard error), P=0.0003]. Spouse controls did not differ from CEPH grandparent controls, literature controls, or controls in a regional epidemiologic study of aging. In a series of 176 autopsy-documented sporadic AD patients, the APOE4 allele was highly significantly associated with AD (0.40±0.026, P=<0.00001). There was no significant association of APOE4 with 16 Mendelian early-onset FAD families that had been linked to APP (CH 21) or CH14 [2,4].

Quantitative vascular and plaque amyloid staining characteristics of AD neuropathology can also be differentiated in patients as a function of APOE genotypes. Congo red staining of vascular amyloid as well as ß-amyloid

immunoreactive plaque density and intensity of staining are increased in brain from AD patients who were E4 homozygotes compared to brain from E3 homozygotes. Correlation of the neuropathologic features with genotype emphasizes the functional association of APOE4 with disease pathogenesis, suggesting further heterogeneity [5].

In order to further define the metabolic role of ApoE in AD, we compared the binding of synthetic βA4 to purified apoE3 and apoE4 [3,6]. Both isoforms bound βA4 peptide forming a complex that resisted dissociation by boiling in sodium dodecyl sulfate. In a series of metabolic experiments, purified apoE4 and apoE3 bound βA4 peptide with differing kinetics; binding to apoE4 was observed in minutes, while binding to apoE3 required hours. In addition apoE4 did not bind βA4 peptide at pH less than 6.6, while apoE3 bound βA4 peptide from pH 7.6 to 4.6. Thus there are differences in the two isoforms with respect to complexing with βA4 peptide. Binding of βA4 peptide by apoE may determine the sequestration or targeting of either apoE or βA4, and isoform specific differences in apoE binding may be involved in the pathogenesis of the extracellular βA4 plaque lesions of AD [6].

Since antibodies to apoE also stain neurofibrillary tangles and the normal metabolism of apoE targets it intracellularly as a lipoprotein-carrier molecule, studies of the intracellular disposition of apoE isoforms and their involvement in neurofibrillary tangle formation are in progress.

These data support APOE4 as a susceptibility gene or genetic risk factor in the pathogenesis of late-onset familial and sporadic AD. ApoE may play a central role in pathogenetic mechanisms leading to neurofibrillary tangles and neuronal death, as well as in amyloid deposition.

References: **(as of 3 March 1993)**

1. Pericak-Vance MA, Bebout JL, Gaskell PC, et al. Linkage studies in familial Alzheimer disease: evidence for chromosome 19 linkage. **Am J Hum Genet,** 1991;48:1034-1050.
2. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: high affinity binding to βA amyloid and increased frequency of type 4 allele in familial Alzheimer's. **Proc Natl Acad Sci USA,** 1993; 90:1977-1981.
3. Roses AD, Pericak-Vance MA, Saunders AM,Schmechel D, Goldgaber D, and Strittmatter W. Complex genetic disease: Can genetic strategies in Alzheimer's disease and new genetic mechanisms be applied to epilepsy? **Epilepsia,** 1993, in press.
4. Saunders AM, Strittmatter WJ, Schmechel D, St. George-Hyslop PH, Pericak-Vance MA, Joo SH, Rosi BL, Gusella JF, Crapper-MacLachlan DR, Alberts MJ, Hulette C, Crain B, Goldgaber D, and Roses AD. Association of apolipoprotein E allele ε4 with late-onset familial and sporadic Alzheimer's disease. **Neurology,** 1993, in press.
5. Schmechel DE, Saunders AM, Strittmatter WJ, Joo SH, Hulette C, Crain B, and Roses AD. Increased vascular and plaque beta-A4 amyloid deposits in sporadic Alzheimer Disease patients with apolipoprotein E4. **Proc Natl Acad Sci USA, 1993,** submitted for publication.
6. Strittmatter WJ, Weisgraber KH, Huang D, Dong L-M, Salvesen GS, Pericak-Vance MA, Schmechel D, Saunders AM, Goldgaber D, and Roses AD. Binding of βA4 peptide to human apolipoprotein E: isoform-specific effects and implications for late-onset Alzheimer's disease. **Proc Natl Acad Sci USA,** 1993, submitted for publication.

ß-PROTEIN IMMUNOREACTIVITY, FIBRILLOGENESIS AND ITS IMPACT ON NEUROPIL AND VESSELS

H.M. Wisniewski, J. Wegiel. Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY, USA

ß-amyloid reactivity is observed in all types of human and animal plaques (diffuse, classical, primitive) meningeal and cortical vessels, lipofuscin and secondary lysosomes, brain trauma, skin, neuromuscular junctions, muscle fibers and some type of neoplastic cells. However, in many of the ß-amyloid immunoreactive areas (some diffuse plaques, secondary lysosomes, skin, neuromuscular junctions) the congo red and thioflavin S staining are negative, indicating that in these areas the ß-immunoreactivity is not associated with the presence of ß-amyloid fibrils. Immunoblotting of diffuse plaques, skin, and areas of brain trauma show several ß-protein immunoreactive peptides including 4kDa band. It may be concluded therefore that not all ß-immunoreactive diffuse plaques or areas will form ß-amyloid fibrils (1,2).

There is no morphological evidence indicating that non-fibrillar deposits of ß-protein damage the neuropil. Structural damage to the neuropil and vessel walls start with the formation of ß-amyloid fibrils. The total area of neuropil occupied by plaques is less than 10% of the volume of the neocortex. However, the impact may be massive depending on localization and the extent of disruption of the communication lines. Studies of the cells (microglia, perivascular cells) associated with deposits of the amyloid fibrils show that intracellular labyrinths of channels connected with extracellular space are the sites of amyloid fibril formation (3,4).

In conclusion our studies support the **membrane hypothesis of amyloid fibril formation.**

References

1. Wisniewski, T., Wisniewski, H.M. Alzheimer's disease and the cerebral amyloidoses. In: Neurodevelopment, Aging and Cognition, edited by I. Kostovic, S. Knezevic, H.M. Wisniewski, G. Spilich, Birkhauser, Boston, pp. 157-172, 1992.

2. Wisniewski, H.M., Wegiel, J., Currie, J.R., Miller, D.L. ß-Protein amyloidosis in Alzheimer's disease. In: Alzheimer's Disease: Advances in Clinical and Basic Research, edited by B. Corain, K. Iqbal, M. Nicolini, B. Winblad, H. Wisniewski, P. Zatta, John Wiley & Sons, Ltd., Chichester, pp. 379-385, 1993.

3. Wisniewski, H.M., Vorbrodt, A.W., Wegiel, J., Morys, J., Lossinsky, A.S. Ultrastructure of the cells forming amyloid fibers in Alzheimer disease and scrapie. Amer. J. Med. Genet. Supp. 7: 287-297, 1990.

4. Wisniewski, H.M., Wegiel, K.C., Lach, B. Ultrastructural studies of the cells forming amyloid in the cortical vessel wall in Alzheimer's disease. Acta Neuropathol. 84: 117-127, 1992.

Studies on APP Biology; Expression and Characterization of a Novel APP Homolog, APLP2, and Analysis of Transgenic Mice Expressing the Entire Human APP Gene. *Sangram S. Sisodia, Hilda H. Slunt, Bruce Lamb, Cornelia Van Koch, John Gearhart, Don Price Departments of Pathology, Neurology, Neuroscience and Obstetrics/Gynecology. The Johns Hopkins University School of Medicine. Baltimore, MD. 21205 USA*

Alzheimer's Disease (AD) is pathologically characterized by the deposition of senile plaques, composed primarily of Aß. Aß are truncated peptides derived from larger amyloid precursor proteins of 695, 751 and 770 amino acids (APP). Neither the function of APP nor the mechanism by which Aß is generated are presently understood. To examine whether APP is a member of a larger gene family, we utilized low stringency hybridization procedures to isolate rodent cDNAs that encode a 751 amino acid polypeptide that is highly homologous APP-751. We show that in rodent tissue, mRNAs encoding the homologue, designated APLP2, are expressed at significant levels in brain, and at higher levels in peripheral tissue. Like APP, APLP2 matures through the secretory pathway, and soluble APLP2-derivatives are secreted. We provide evidence that a wide spectrum of antibodies generated against APP are unable to discriminate between APP and APLP2, arguing that earlier studies which purported to identify APP by immunochemical criteria may have followed APLP2, as well. We speculate that cellular expression and maturation of APLP2 and APP are critically balanced, and that subtle alterations of this stoichiometry may predispose APP to be diverted into pathways intermediary to the formation of Aß. In order to develop an animal model that mimics the pathological features of AD, we have used yeast artificial chromosome (YAC)/ embryonic stem (ES) cell technology to generate transgenic mice which harbor the entire human APP gene. We will present data on the tissue specific expression of transgene-encoded products, and discuss the potential of this methodology to address mechanisms of Aß amyloidogenesis.

PROCESSING AND BIOLOGICAL ACTIVITY OF APP

Nikolaos K. Robakis, Spiros Efthimiopoulos, Kumar Sambamurti, Dido Vassilacopoulou, Lawrence M. Refolo, James A. Ripellino, and Junichi Shioi

Amyloid β protein (β/A_4 or Aβ) derives from the proteolytic processing of the amyloid precursor protein (APP), which is cleaved by at least three different secretase activities (I, II and β-secretase). Secretase activity II cleaves APP after Aβ Lys-28 and may produce the C-terminus of the amyloid peptide.[1] Full length intracellular APP is cleaved by secretases either in the trans-Golgi network or in post-Golgi vesicles. The cleaved APP is transported to the cell surface in vesicles.[2] In certain cell lines a fraction of full length APP reaches the cell surface uncleaved. Treatment of these cell lines with agents that inhibit endocytosis (methylamine, colchicine, reduced temperature) inhibited secretion of the cell surface APP suggesting that endocytosis is required for the secretory cleavage of this APP. The endocytosed APP may be transported to the trans-Golgi network where it is cleaved by APP secretases. Protein kinase C seems to regulate the activity of secretases, and phorbol esters increase the levels of secreted APP. The construction of several fusion APP proteins showed that extracytoplasmic APP sequences are important for the regulation of the secretory cleavage of cell surface APP by phorbol esters while the cytoplasmic domain of APP is important for the secretion of both cell surface and intracellular APP.

The biological activity of the APP is still not completely understood, although it seems to act as a cell adhesion molecule. Recent studies have shown that in glioma cells, most of the soluble secreted APP and cell surface APP occur as a chondroitin sulfate proteoglycan (CSPG).[3] In addition, full length APP CSPG has been detected in numerous other cell lines including neuroblastoma and fibroblast cells and in human and rat brain tissue. These results suggest that the proteoglycan nature of the APP proteins may be important for their biological function. An altered biological activity of APP may be involved in the development of familial Alzheimer's disease (FAD) in patients with mutations on the APP gene.[4]

1. Anderson et al., (1992). J. Neurochem. 59, 2328-2331.
2. Sambamurti et al., (1992). J. Neurosci. Res. 33, 319-329.
3. Shioi et al., (1992). J. Biol. Chem. 267, 13819-13822.
4. Goate et al., (1991). Nature 349, 704-706.

REGULATION OF NEURONS AND ASTROCYTES BY PROTEASE NEXIN-1 AND THROMBIN: POSSIBLE SIGNIFICANCE IN INJURY, INFLAMMATION AND ALZHEIMER'S DISEASE. Dennis D. Cunningham. Department of Microbiology and Molecular Genetics, University of California, Irvine, CA 92717

Protease Nexin-1 (PN-1) is a 43 kDa protease inhibitor that is abundant in brain and secreted by astrocytes. In solution it rapidly inhibits thrombin, urokinase and plasmin by forming a stable complex with the catalytic site serine of the protease. The complexes bind back to the astrocytes and are rapidly internalized and degraded. This provides a localized mechanism for inhibiting and clearing these proteases from the extracellular environment. This localized control is appropriate for the brain because the blood brain barrier prevents plasma protease inhibitors from entering the brain.

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 and neurons. 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 similary regulate processes on cultured astrocytes. Thrombin is a potent mitogen for cultured astrocytes; this activity can be modulated by cell-secreted or added PN-1. The cellular signalling mechanism by thrombin for both process retraction and mitogenic stimulation involves a receptor that is quite similar to the one recently cloned from platelets.

Prothrombin mRNA is present in brain and in cell lines of neuronal and glial origin, suggesting that thrombin may be synthesized in the brain. Thus, the regulation of neurons and astrocytes by thrombin,

observed in cell culture, might play a role in certain developmental or regulatory events *in vivo* although this has not yet been demonstrated. The results presented below suggest that the actions of thrombin and PN-1 on neurons and astrocytes might be important in certain pathological conditions.

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 contribute to regulation of blood coagulation and limit deleterious effects of thrombin on neurons and glial cells.

REFERENCES

Neurite Outgrowth Activity of Protease Nexin-1 Requires Thrombin Inhibition. David Gurwitz and Dennis D. Cunningham. Journal of Cellular Physiology 142, 155 (1990).

Reciprocal Modulation of Astrocyte Stellation by Thrombin and Protease Nexin-1. Kathleen P. Cavanaugh, David Gurwitz, Dennis D. Cunningham and Ralph A. Bradshaw. Journal of Neurochemistry 54, 1735 (1990).

Prothrombin mRNA is Expressed by Cells of the Nervous System. Melitta Dihanich, Matthew Kaser, Eva Reinhard, Dennis Cunningham and Denis Monard. Neuron 6, 575 (1991).

Regulation of Protease Nexin-1 Synthesis and Secretion in Cultured Brain Cells by Injury-related Factors. Patrick J. Vauahan and Dennis D. Cunningham. Journal of Biological Chemistry, 268, 3720-3727 (1993).

The early senile plaque: a reappraisal

A. Probst, D. Langui, B. Landwehrmeier and J. Ulrich.

Institute of Pathology, University of Basel,
4003 Basel, Switzerland

Senile plaques (SP) are among the most salient features found in the brains of elderly individuals and in patients with Alzheimer's disease and Down's syndrome. Although heterogeneous in composition, all subtypes of SP contain a ubiquitous constituent called the ß/A4 protein, which derives from a precursor protein, the amyloid precursor protein (APP). The classical senile plaque is a complex lesion containing a central core of compacted amyloid surrounded by dystrophic neuritic processes, activated microglial cells and reactive astrocytes.Recent histochemical and immunohistochemical studies have revealed important new information on the pathogenesis of SP by drawing attention to a special type of SP which essentially consists of diffuse deposits ("diffuse plaques") of ß/A4 in a non fibrillar (Thioflavine negative) conformation and which develop in an apparently normal neuropil, lacking obvious dystrophic neurites or reactive glia. Diffuse ß/A4 deposits are the most prevalent type of plaque detectable in elderly patients without dementia and have been described in the absence of typical plaques in the brain of individuals with Down's syndrome (Tagliavini et al 1988; Giaccone et al 1989). This has led to the conclusion that diffuse plaques represent SP at a very early stage of development and that dystrophic neurites or reactive glia are not required for ß/A4 deposition.

The origin of the extracellular ß/A4 found in senile plaques, especially in its putative early type (diffuse plaque), and the cellular mechanisms responsible for its deposition in cerebral tissues, continue to be a highly controversial issue.

In a recent investigation (Papolla et al 1992) using computerized image analysis and quantitative immunohistochemistry, evidences arguing for a neuronal origin of ß/A4 were obtained.The presence of a neurocentric concentration of ß/A4

protein accumulation was demonstrated. Furthermore, computerized image analysis showed patterns that can be interpreted as a pathogenic sequence ranging from initial neurogenic concentration gradient centered around one single neuron to larger deposits (the diffuse plaques) arising by anastomosing gradients around several neurons.

Using immunohistochemistry on serial paraffin sections, we analyzed in detail the distribution of ß/A4 in diffuse plaques in a population of intellectually intact elderly individuals. Discontinuous dot-like or horseshoe-shaped ß/A4 immunoreactive deposits were found along dendrites, and around the soma of neurons included in the plaques. Furthermore increased synaptophysin reactivity with slightly dilated synaptophysin-immunolabelled presynaptic terminals were found in diffuse plaques. APP epitopes could not be found in diffuse plaques. However some of the APP antibodies, mainly those to the C-terminal portion of APP, and antibodies to ß/A4, recognized clusters of flat vesicular profiles (0.6-1.4µm in width and 2-3µm in length) in the neuropil of cortical areas where plaques had developed. Our findings are compatible with a neuronal origin of ß/A4 in diffuse plaques and with a primary release of ß/A4 at synaptic sites along neurites. They also suggest that diffuse plaques might be preceded by minute lesions of the neuropil where ß/A4 is not yet released from the precursor molecule.

A second controversial issue is whether ß/A4 deposition follows or triggers neuronal degeneration. Little morphological evidence for a primary defekt in neurites, as a nidus for accumulation of ß/A4 in tissues, is presently available. Abnormal neurites have been reported in aged monkeys and ß/A4 visualized in their close vicinity, suggesting that neuritic abnormalities may be one focus for the release of ß/A4 (Cork et al.1990). That ß/A4 is able to induce alterations in neurons, when present in critical amounts in the neuropil, is suggested by various observations.Although controversial,in vitro studies argue for a dose-dependent neurotoxic effect of ß/A4 (Yankner et al 1989;1990;Koh et al.1990) Studies in normal aged and Down's

syndrome patients have shown that ß/A4 deposition precedes the development of dystrophic neurites (Tagliavini et al 1988; Giaccone et al 1989). The presence of abnormally ubiquitinated (tau-negative) neurites has been documented in early (preamyloid) plaques in hereditary cerebral hemorrhage with amyloidosis Dutch type (HCHWA-D) and Alzheimer's disease (Buggiani et al 1990). Although latter data might be interpreted in the sense of a primary pathology of neurites, indirect evidence, e.g. the higher incidence of ubiquitinated neurites in association with amyloid than with preamyloid deposits, suggests the opposite. We recently investigated serial sections of various neo and allocortical areas from 5 patients with Alzheimer's disease using an anti-ß/A4 12-28 antibody, a monoclonal antibody to hyper-phosphorylated epitopes of tau protein (PHF-1, Dr. Greenberg),and thioflavin S, modified Bielschowsky and Gallyas stains. In the hippocampus, PHF-1-positive material was identified in the majority of diffuse plaques, where it appeared to be associated with morphologically normal thioflavin S-negative neurites. Most of these plaque-like accumulations of PHF-1-positive neurites were not evident in Gallyas or modified Bielschowsky stained sections, indicating that in early stages of accumulation, hyperphosphorylated tau protein is probably not yet in the shape of PHF. In some hippocampal areas, tau-positive neurites were seen in diffuse plaques even in the absence of threads and tangles in the surrounding cortex. In contrast diffuse neocortical plaques did not contain tau-positive neurites in the absence of threads and tangles in local pyramidal neurons.

These results suggest that accumulation of abnormally phosphorylated tau protein is an early event preceding the degeneration of neurites in senile plaques. Present evidence suggests that this accumulation may, in some way, be triggerd by local events associated with ß/A4 deposition already at early stages of plaque formation.

Binding of the Dye Congo Red to the Amyloid Protein Pig Insulin Reveals a Novel Homology amongst Amyloid-forming Peptide Sequences

William G. Turnell† and John T. Finch

MRC Laboratory of Molecular Biology
Hills Road, Cambridge, CB2 2QH, U.K.

(Received 19 December 1991; accepted 2 July 1992)

The three-dimensional structure has been determined of a complex of the dye Congo Red, a specific stain for amyloid deposits, bound to the amyloid protein insulin. One dye molecule intercalates between two globular insulin molecules at an interface formed by a pair of anti-parallel β-strands. This result, together with analysis of the primary sequences of other amyloidogenic proteins and peptides suggests that this mode of dye-binding to amyloid could be general. Moreover, the structure of this dye-binding interface between protein molecules provides an insight into the polymerization of amyloidogenic proteins into amyloid fibres.

Thus the detailed characterization, at a resolution of 2·5 Å, of the dye binding site in insulin could form a basis for the design of agents targeted against a variety of amyloid deposits.

Keywords: amyloid; insulin; Congo Red; protein structure; crystallography

Animal Models

ANIMAL MODELS OF ALZHEIMER'S DISEASE

Donald L. Price, M.D.

Sangram S. Sisodia, Ph.D.

Lary C. Walker, Ph.D.

Cheryl A. Kitt, Ph.D.

Bruce T. Lamb, Ph.D.

and

John D. Gearhart, Ph.D

From the Departments of Pathology (DLP,SSS,CAK), Neurology (DLP), Neuroscience (DLP), Gynecology & Obstetrics (BTL,JTG), and Physiology (BTL,JTG) and the Neuropathology Laboratory (DLP,SSS,LCW,CAK) and Developmental Genetics Laboratory (BTL,JTG), The Johns Hopkins University School of Medicine, Baltimore, Maryland, U.S.A

It is difficult to reconstruct the character, evolution, and mechanisms of cellular abnormalities in end-stage human disease. To clarify some of these issues, investigators have turned to studies of animal models that share features in common with human diseases. This review focuses on investigations of rats, aged nonhuman primates, and transgenic mice as models of Alzheimer's disease (AD).

Studies of APP Biology in Control Animals

The APP gene is spliced alternatively to generate

transcripts that code for proteins of 695, 751, and 770 amino acids. APP are cleaved within the β-amyloid protein (Aβ) domain by the enzyme α-APP secretase; this process releases a large portion of the ectodomain of APP. Proteolysis at the secretase site prevents the formation of Aß. Neurons express all three APP transcripts, and APP are synthesized in cell bodies and proximal dendrites. APP-695 is the most prominent neuronal isoform. Neuronal APP are delivered to axons and terminals via the fast anterograde transport system. Because APP are delivered to nerve terminals, the protein is thought to play a role in synaptic interactions. To date, relatively little is known about the trafficking and processing of APP at presynaptic sites. Our recent studies suggest that APP may be cleaved at terminals and that the cleavage site is within Aß, an event that would prevent the formation of Aß. However, some APP may also be processed by alternative pathways that could generate amyloidogenic C-terminal fragments (i.e., peptides that contain the Aß C-terminal domain of APP). Alterations in the cleavage patterns of APP presumably increase the formation of Aß.

Investigations of Aged Nonhuman Primates

At present, the best available model for research relevant to AD are aged *Macaca mulatta*, which have a life span of >30 years (equivalent to 90 years in humans). At the end of the second decade of life, these animals develop age-associated

impairments in performance on cognitive and memory tasks, as well as brain abnormalities, including the formation of neurites and the deposition of Aß. Neurites (i.e., enlarged axons, nerve terminals, and dendrites), filled with abnormal membranes, dense bodies, lysosomes, cytoskeletal elements, and abnormal-appearing mitochondria, are derived from cholinergic, monoaminergic, serotoninergic, γ-aminobutyric acid (GABA)ergic, and peptidergic neurons. Modest reductions in cholinergic, monoaminergic, and certain peptidergic markers have been detected in the brains of old animals, but these deficits never reach the severity documented in the brains of cases of AD. Late in the second decade and throughout subsequent years, many animals also show the presence of Aß in diffuse deposits, in the cores of senile plaques, and in the walls of blood vessels. Although the sources of Aß are not fully established, neurons that transport APP to nerve terminals and process proteins at these sites appear to be one obvious source. Significantly, in aged monkeys, Aß is readily demonstrable in proximity to swollen APP-enriched neurites. At these sites, APP are presumably not clipped at the α-APP secretase site but, instead, are cleaved upstream to the N-terminal position of Aß to generate potentially amyloidogenic fragments. These C-terminal peptides could then be further processed to form Aß, which then decorates APP-containing neurites. Other cells, including astrocytes and microglia, are present at these sites and may contribute to the formation of Aß.

We have speculated that APP may play an important role in synaptic functions, perhaps by maintaining synaptic interactions. Thus, alterations in APP processing could lead to altered functions at synapses, including synaptic disjunction, a potentially reversible process, followed by irreversible synaptic disconnection. Subsequently, disconnected axons die back, and retrograde abnormalities, similar to those that occur following axotomy, appear in cell bodies. Eventually, cytoskeletal elements in perikarya become severely perturbed, leading to the formation of neurofibrillary tangles. Finally, affected neurons die. This hypothetical scenario is compatible with studies of individuals with AD who show alterations in synapses.

Studies of Transgenic Mice

Several groups have begun to make transgenic mice to produce a small animal model of AD. To date, these efforts have not been very successful, in part, because of low levels of transgenes and protein products and because of some misinterpretation of studies of brain abnormalities in these animals. Recently, a 650-kb yeast artificial chromosome (YAC), which contains the entire unrearranged APP gene within 400 kb, was transferred into embryonic stem (ES) cells by lipid-mediated transfection. ES lines were isolated that had stably integrated the human APP gene in an unrearranged state and that expressed properly initiated and spliced full-length human APP mRNA. The line that produced

the highest level of human APP RNA also produced human protein. Chimeras generated from two ES lines have transmitted the YAC to their offspring, generating novel APP YAC transgenic mice. These transgenic mice express human APP mRNA and protein at significant levels in brain and peripheral tissues that mirror the expression of endogenous mouse APP products, including the regional-specific expression of human APP in the brain of a three-month old transgenic animal. Thus, these mice mimic the dosage imbalance observed in individuals with Down's syndrome. If APP overexpression is a critical event in amyloidogenesis associated with Down's syndrome, then we anticipate that these animals will develop Aß in adult life. Moreover, no group has yet reported transgenic mice with missense mutations at position 717 or at positions 670 and 671 (Swedish double mutation) of APP that are linked to the presence of early-onset autosomal dominant AD. The YAC-ES transgenic technology can be used to introduce APP genes into these mutations. These mice, investigated by strategies similar to those used in aged monkeys, will serve as excellent models to clarify the mechanisms of amyloidogenesis and to test antiamyloidogenic therapies *in vivo*.

NUCLEATION OF AMYLOIDOGENESIS IN INFECTIOUS AND NONINFECTIOUS AMYLOIDOSES OF BRAIN

C. Gajdusek, National Institutes of Health, Bethesda, MD. USA

Our primary area of inquiry continues to be elucidation of the pathogenesis of dementing brain amyloidoses, both the transmissible type (such as kuru, Creutzfeldt-Jacob disease (CJD), Gertsmann-Sträussler syndrome (GSS), fatal familial insomnia (FFI), scrapie, and bovine spongiform encephalopathy (BSE) caused by infectious amyloidoses) and the nontransmissible type (such as normal aging, Alzheimer's disease (AD), and Down's syndrome). We are now concentrating on the protein chemistry and crystallography of the configurational change of host precursor protein to the infectious amyloid form both *in vivo* and *in vitro*. Our program continues to characterize the molecular basis for the genetic control of *de novo* generation of infectious amyloid proteins from host precursors in kuru, CJD, GSS, FFI, scrapie, and BSE.

We are also concentrating on the phenomenon of nucleation with the induction of patterned configurational change. Many proteins in the amyloid β-pleated configuration induce other amyloidogenic molecules to form amyloid by this process, which we call "induction of configurational change" and which others call "instructional amyloidogenesis." We have for long believed that the amyloid enhancing factors (amyloid augmenting factors) are really scrapie-like agents nucleating the configurational change to amyloid, as do amyloid fibers themselves (fibril amyloid enhancing factors). The polymer chemistry of nucleation and of fibril polymerization has much to teach us in this field which will be applicable to studies of amyloidogenesis in AD, aging brain, and in the transmissible brain amyloidoses (kuru-CJD-GSS-FFI-scrapie-BSE).

Many laboratories are now pursuing the problem of the unconventional viruses, which are replicating proteins, to determine how

the amyloid precursor protein is converted to an infectious form by configurational changes in the tertiary and quaternary structure of the normal precursor. On inoculation of susceptible hosts, the CJD or scrapie amyloid monomer autonucleates and autopatterns this conversion of the normal, noninfectious host precursor into the infectious form. We believe that most sporadic cases of CJD arise by *de novo* spontaneous conversion of the normal precursor to the infectious form, a rare event occurring at the frequency of one per million persons per year (the annual incidence of CJD throughout the world). In the familial forms of CJD, GSS, and FFI, in which the occurrence is an autosomal dominant trait, we have found that each family has one of several different mutations, 7 causing a single amino acid change and another causing the change of the codon to a stop codon. Familial CJD, GSS, and FFI is also caused by the insertion of 2, 5, 6, 7, 8, 9, or 10 octapeptide repeats. Each mutation causes a million-fold increased probability of the spontaneous configurational change to an infectious polypeptide, and appears as an autosomal dominant trait. Thus, the behavior of the transmissible brain amyloidoses parallels completely that of the transthyretin amyloidoses causing familial amyloidotic polyneuropathy, in which there are more than 30 point mutations, each of which enormously increases the likelihood of a configurational change of transthyretin to an amyloid in different families.

Several point mutations cause single amino acid substitutions which lead to distinct different forms of CJD, GSS, or FFI differing in clinical course, incubation period, duration, EEG changes, distribution of lesions, and presence of amyloid plaques. Incubation period on first passage in primate hosts may vary for different mutations. Yet, on

second passage into primate hosts, the differences disappear, the passaged virus all being the same chimpanzee infectious amyloid with the normal chimpanzee amino acid sequence.

Our discovery that the codon 200 point mutation (glutamic acid to lysine) causes the CJD in the two high-incidence foci of CJD in the Orava and Lucenec regions of Slovakia has been followed by our demonstration that this same point mutation is linked to CJD cases in Eastern Europe from Poland through Greece, and even in cases in the United States and in South Americans of Eastern European Slavic origin. This has led to our quickly discovering that this same point mutation underlies the high incidence of CJD in Sephardic Jews of Libyan origin, both in immigrants to Israel and Israeli-born. Furthermore, we have found this same mutation in Sephardic Jews with CJD from Tunis and Greece and thus, it is a circum-Mediterranean Jewish trait as well as of Eastern European Slavs. Since the Jews, with the Arabs, moved across Northern Africa to the Iberian Peninsula, and, in the late 15th century, fled as Sephardic Jews from Spain to Greece and elsewhere in the Easter Mediterranean, this codon 200 mutation has proved to be a marker of the "Wandering Jew of the Diaspora." We have shown that this codon mutation 200 also underlies the high incidence of familial CJD in Chile. We have also traced throughout Europe and the Americas a codon 178 mutation (aspartic acid to asparagine) which causes a rapidly progressive form of CJD.

Fortunately, from our CJD transmission work spanning 30 years we know that most of the mutations, including the octapeptide repeat inserts, produce infectious amyloids which are transmissible to susceptible laboratory animals, some with incubation periods lasting

more than 10 years. We have transmissions in monkeys or chimpanzees from CJD cases with each of the point mutations or inserts, and the affected primates produce infectious amyloids which do not contain these point mutations of insertions.

The possibility that a synthetic polypeptide containing the precursor protein sequence with codons 102, 178, and 200 mutations, or the octapeptide inserts themselves, might be infectious and serve to nucleate the autopatterned configurational change in the host precursor protein to its infectious form is being investigated by inoculation of many species, including susceptible monkeys, with such synthetic polypeptide. Finally, we are also investigating the possible infectiousness of baculovirus-expressed peptides of the human CJD amyloid containing these and other mutations.

We continue to insist that normal brain aging and Alzheimer's disease (AD) are the same type of nontransmissible amyloidosis, with different speeds of conversion of the βA4 amyloid precursor protein to amyloid fibrils. Environmental, nongenetic, probably toxic causes surely influence the amyloid conversion in sporadic AD. However, in some families with familial AD, there is one of several point mutations on the βA4 amyloid precursor protein at codon 717 (valine to isolensine, phenolatine, or glycine), and in families with Dutch hereditary cerebral hemorrhage, a different point mutation at codon 613 (glutamic acid to glutamine). In the latter, the point mutation is expressed only in endothelial cells producing β-amyloid fibrils around vessels, but no amyloid plaque cores or amyloid in neurofibrillary tangles. We were the first to characterize the gene for this aging brain amyloid precursor protein (βA4 protein), to locate it on chromosome 21 of man and 16 of

mouse, and to show its high evolutionary conservation. This gene is identical to that for an excreted rapidly turned-over protein (protease nexin II) which specifically binds to the subunit of nerve growth factor and many other serine proteases and cell modulators which contain such serine protease as binding regions. This important negative feedback control loop may explain the rapid synthesis, short half-life and wide distribution in neurons of this brain amyloid precursor. Our work showing the gene was expressed in several alternatively spliced forms, with and without a 57 bp or 76 bp insert which specifies a serine protease inhibitor, now fits will with the identity of protease nexin II and the alternatively spliced forms of our amyloid precursor protein containing the serine protease inserts.

Our studies of amyloid β-protein mRNA expression in different brain cells of normal juveniles, aging brain and AD brains have revealed that all neurons which develop neurofibrillary tangles (NFT) and are most vulnerable to loss in aging, and in AD, express a very high level of turned-on message. However, not all cells with high levels of amyloid β-protein mRNA develop NFT formation, and thus its high expression appears to be a necessary, but not sufficient, condition for NFT formation. Interestingly, in Guamanian amyotrophic lateral sclerosis (ALS) and parkinsonism dementia (PD), typical NFT appear in large motor neurons which contain a high, up-regulated mRNA for amyloid β-protein. We have also studied regulation of mRNA and precursor protein expression in hippocampal neurons and in endothelial cells *in vitro*. Thus, continued molecular and cell biology studies of brain amyloid β-protein biosynthesis, processing, and regulation will surely continue to dominate research on AD and aging brain for some time.

The discovery that the subunit of aging brain amyloid in AD and Down's syndrome had no amino acid homology with the much larger monomer of scrapie-associated fibrils (SAFs) or kuru plaques led to the clear differentiation of transmissible from nontransmissible brain amyloidoses. Scrapie and the transmissible dementias (kuru, CJD, and its GSS and FFI variants) form the amyloid of SAF and scrapie or kuru plaques from a proteolytically cleaved portion of the larger infectious form of the scrapie amyloid precursor protein. The genes for aging brain amyloid precursor and for the scrapie amyloid precursor show no sequence homology and the amyloidogenic subunits cleaved from the full-length precursors are extremely different. The subunit protein for the nontransmissible brain amyloidoses of aging and AD is a polypeptide of 4.1 kDa (42 amino acids) in size and is nonglycosylated while that from scrapie-kuru-CJD is 27 kDa in size and has two glycosylated sites. In humans, the genes for the aging brain amyloid precursor and for the scrapie amyloid precursor are on chromosomes 21 and 20, respectively.

POSTERS

A decrease in serum sialyltransferase levels in Alzheimer's disease

T.M. Maguire[1], A.M. Gillian[1], D.O'Mahony[2], A. Dennihan[1] and K.C. Breen[1,3]

[1]Dept. of Pharmacology, University College, Dublin, Ireland, [2]The Mercers Institute for Research on Ageing, St. James's Hospital, Dublin 6, Ireland, and [3]Dept. of Pharmacology and Clinical Pharmacology, University of Dundee, Scotland, U.K.

There is some evidence for an abnormality in the functioning of the hypothalamic-pituitary-adrenal (HPA) axis in a subsection of patients suffering from Alzheimer's disease (AD). A positive correlation has been observed in some AD patients between non-responsiveness to the dexamethasone suppression test (DST) and both the severity of dementia and memory ability. An altered serum steroid level may subsequently play a role in the accelerated degeneration of neural cells in certain brain regions. Previous studies have demonstrated a steroid-dependent release of a soluble form of the sialyltransferase (STase) enzyme from liver cells. It is possible, therefore, that a measure of serum STase activity may be used as an indication of steroid receptor function when compared with serum steroid levels.

In this study, we have noted an age-related increase in serum cortisol levels in elderly control subjects which was blunted in a group of age-matched AD patients. There was also a significant decrease in serum levels of STase activity in the AD group when compared with both elderly (over 60 years) and young (under 60 years) controls. In a population of Down's syndrome patients, there was an age-related decrease in serum STase activity in patients from 20 to 50 years to levels similar to those observed in the AD group. In the elderly (over 60 years) control group, there was a direct correlation between elevated serum cortisol levels and STase activity. This correlation was not observed in any other subject group. The significant decrease in serum sialyltransferase levels observed may thus provide a useful peripheral biochemical marker of neurodegeneration.

This work was supported by The Health Research Board of Ireland, EOLAS and the Sandoz Foundation for Gerontological Research. KCB was supported by a H.J. Heinz Newman Scholarship.

STUDY WITH PEROXIDASE (HRP) OF THE CONTRALATERAL PROJECTIONS OF THE NEOCORTEX IN AGED ALBINO RATS.

García-Méndez, J.A.; Lanciego, J.L. & F. Collía
Dpt. of Human Anatomy & Histology. Histology. Fac. of Medicine. Univ. Salamanca

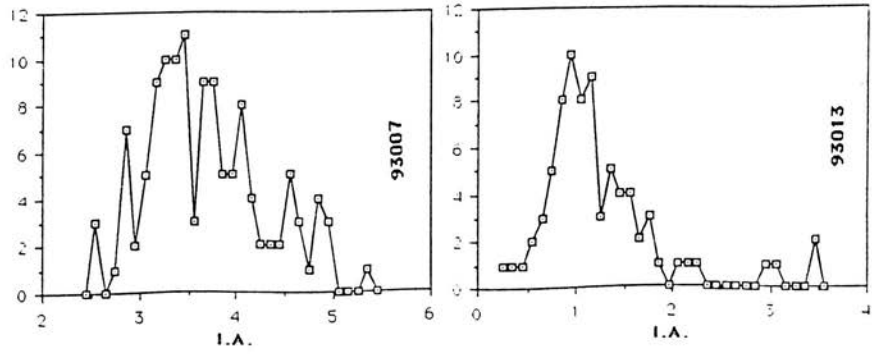
The aim of the present study was to identify the topographic location of the neurons of the neocortex projecting to the auditory (Te 1, te 2 and Te 3) and contralateral visual (Oc 1) areas. Ten albino rats of both sexes with ages ranging between 24 and 42 months received iontophoretic injections of HRP (Sigma type VI) in different areas of the temporal and adjacent occipital cortex. After a tracing time of 48 hours the animals were perfused and processed according to the technique of Mesulam. Microscope study of the serial coronal sections obtained was completed by making schematic drawings of them with the help of a drawing tube, depicting in them the inequivocally labelled neurons in the contralateral cortex and making schematic representations of the individual extensions of the projections.

The contralaterally labelled neurons showed a maximum frequency in a homotopic territory and were grouped in other heterotopic areas that varied in location, depending on the site of injection.

The comparative study of the results obtained in aged rats partially coincides with those obtained in adult rats, although some quantitative differences exist (relatively fewer labeled neurons in the aged animals).

Fig 1.- Case 93007. On the Y axis, labeled neurons in a case injected in the center of the primary auditive cortex (Te 1) centered at coordinate IA: 4.00 mm to 3.50 mm above the rhinal fissure.

Fig 2.- Case 93013. Labelled neurons in a case injected in the associative auditory cortex (Te 2) also affecting the visual cortex (Oc 1) with a rostrocaudal coordinate IA: 1.30 mm to 1.70 mm above the rhinal cortex.



Supported by Spanish Commission of Science and Technology CICYT nº SAF-248/92.

ENZYMATIC DIGESTION PATTERNS OF THE AMYLOID PROTEIN PRECURSOR IN THE CSF OF PATIENTS WITH ALZHEIMER'S DISEASE

F Coria, A Moreno, I Rubio, MA García
Dept. of Neurology, Neurological Research Laboratory, Segovia general Hospital. Segovia, Spain

Alzheimer's disease (AD) is characterized by the deposition in the brain of an amyloidogenic polypeptide of 39-42 amino acids, called β-protein. This fragment arises by proteolytic cleavage of a larger protein, called amyloid protein precursor (APP). Recent studies suggest that release of β-amyloid is favoured by APP mutations identified in some inherited cases of early-onset AD. To test whether abnormalities of this protein may also influence this proteolytic event in sporadic AD cases, we compared the enzymatic digestion patterns of APP from patients with probable AD (NINCDS-ADRDA criteria) and controls, using a modified version of the method developed by Cleveland et al. CSF samples obtained by lumbar puncture were digested with V8 protease for 30 min. at 37 ^{o}C, separated by SDS-PAGE and transferred to PVDF membranes. The resulting peptides were identified with a panel of antibodies against several domains of the APP molecule. Under the experimental conditions used, V8 protease completely digest CSF-APP and releases a number of high and low MW peptides from different regions of the molecule. Qualitative and quantitative analysis of these digestion patterns disclosed no differences between patients and controls. Further studies in familial and non-familial cases of AD, using proteolytic enzymes of different specificities are underway in an attempt to identify possible pathogenetic abnormalities of APP in this disorder.

LEVELS OF AMYLOID PROTEIN PRECURSOR IN THE CSF OF PATIENTS WITH ALZHEIMER'S DISEASE

F Coria, A Moreno, I Rubio, MA García
Dept. of Neurology, Neurological Research Laboratory, Segovia general Hospital, Segovia, Spain

Alzheimer's disease appears to be caused by the deposition of an amyloidogenic proteolytic fragment of a protein called amyloid protein precursor. This protein can be detected as several isoforms in several tissues and body fluids, including plasma and CSF. In order to know whether abnormal depsition of APP fragments in AD brains are reflected in CSF, we measured the levels of CSF-APP in AD patients at different stages and controls. CSF samples from patients with AD, neurologically impaired and normal controls were separated by PAGE under non-reducing conditions, transferred to PVDF membranes, and detected with a antibodies which recognized the entire APP molecule. The levels of APP were determined by laser scanning densitometry. We found that independently of the antibody used, the levels of APP in AD patients and controls overlapp considerably, and no statistical differences could be found among the three groups of patients tested. However, when APP levels were plotted against neuropsychological scores of AD patients a significant inverse linear correlation was observed. These data suggests that the levels of APP in the CSF correlates with the degree of dementia in AD. This could reflect loss of neurons and other APP producing cells during the course of this disease.

AMYLOID BETA-PROTEIN STIMULATES CASEIN KINASE I AND CASEIN KINASE II ACTIVITIES

Abha Chauhan, Ved P.S. Chauhan, N. Murakami, H. Brockerhoff and H.M. Wisniewski. New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, New York 10314.

Amyloid beta-protein (Aß), the major protein of cerebrovascular and plaque amyloid in Alzheimer disease, is considered a primary factor in the pathology of this disease. Both neurotrophic and neurotoxic effects have been described for Aß depending on the neuronal age and the concentration of Aß, but the biochemical consequences of Aß accumulation remain unknown. We have previously reported a remarkable homology in the amino acid sequence of hydrophobic region of Aß and viral fusion proteins, and decrease in the fluidity of membranes by Aß 1-40. Based on these observations, we have postulated that Aß in the soluble form may affect membrane properties and begin to modify the structure and function of the membrane in which it is embedded by changing membrane permeability or enzyme or receptor activity. The likely targets for such change are protein phosphorylating enzymes (protein kinases) which are highly concentrated in the brain. Extensive evidence has demonstrated abnormal protein phosphorylation in Alzheimer's disease. Recently we reported that synthetic Aß 1-40 alters Protein kinase C (PKC) - mediated protein phosphorylation (Life Sci. (1991) 49, 1555-62). In the present study, we investigated the effect of synthetic Aß with the amino acid sequence corresponding to cerebrovascular Aß and plaque Aß on Casein kinase I (CK I) and Casein kinase II (CK II). Casein kinases are messenger - independent kinases that play an important role in the control of variety of cellular processes. CK I and CK II were purified from bovine brain by column chromatography on phosphocellulose, and casein was used as a substrate. Aß was found to stimulate markedly the activities of both kinases in a concentration-dependent manner. The effect of plaque Aß was considerably higher than that of cerebro-vascular Aß. Heparin, which is known to be a specific inhibitor of CK II, completely inhibited Aß-stimulated CK II activity. Aß itself was not a substrate for casein kinases. These findings were confirmed using other substrates for CK I0 and CK II. The experiments with synthetic CK II - specific substrate peptide (Leu-Glu-Leu-Ser-Asp-Asp-Asp-Asp-Glu), and the phosphorylation of erythrocyte-membrane proteins by intrinsic membrane-bound CK I in erythrocytes showed marked stimulation in activities of casein kinases in presence of Aß. We propose that Aß by stimulating Casein kinases may contribute to abnormal protein phosphorylation in Alzheimer's disease, in particular to increased phosphorylation of microtubule-associated proteins, leading to the neurofibrillary tangles formation and neurodegeneration in this disease. Interaction of Aß with protein kinases thus may characterize the beginning of the disease.

SYNAPSE LOSS FROM THE HIPPOCAMPUS IN ALZHEIMER'S DISEASE.

D. C. Davies and A. Bhalla. Dept. of Anatomy, St. George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE, U. K.

Memory deficits in Alzheimer's disease (AD) patients are often far more severe than the amount of gross pathology (senile plaques, neurofibrillary tangles, neuronal loss) in the brain would suggest. We have therefore investigated the integrity of synaptic connections in AD hippocampus in an attempt to determine whether neuronal connections are more severely disrupted than histopathology would suggest.

Left hippocampi were obtained from 6 histopathologically confirmed AD and 10 neurologically normal aged brains fixed in a modified Karnowsky solution. Tissue was microdissected from four subfields of the hippocampal formation: the dentate gyrus, CA3, CA1 and the subiculum, and processed for electron microscopy. Electron photomicrographs were taken of the the principal synaptic fields in theses region asymmetrical and symmetrical synapses identified, counted and their apposition zone lengths measured.

The surface density of asymmetric synapses was significantly reduced in the molecular layers of CA1 (73%), CA3 (62%), subiculum (47%) and the inner part of the molecular layer of the dentate gyrus (35%) in AD hippocampi compared with controls. There was no significant difference in the surface density of symmetric synapses in AD hippocampi compared to controls. There was a significant 17% increase in the length of remaining asymmetric synapses in the CA1 and CA3 of AD hippocampi compared to controls. There was no effect of the disease on symmetric synapses.

These results show that synapse loss is more severe than other markers of AD in the hippocampus e.g. neuronal loss and is more widespread, occurring in regions not usually affected by overt pathology. Moreover, asymmetric, probably excitatory synapses appear to be preferentially affected. The increase in size of some remaining asymmetric synapses suggests that they may be showing a plastic response to the disease process.

DIFFERENTIAL BRAIN AREA VULNERABILITY TO LONG TERM SUBCORTICAL EXCITOTOXIC LESIONS N. Mahy, G. Bendahan, M.Ll. Boatell, B. Bjelke*, B. Tinner,* L. Olson* and K. Fuxe*. Unity of Biochemistry, School of Medicine, University of Barcelona, Barcelona, Spain, *Department of Histology and Neurobiology, Karolinska Institutet, Stockholm, Sweden

Microinjections of quisqualic acid and ibotenic acid were made into the medial septal nucleus and the ventral part of globus pallidus of adult male Sprague-Dawley rats. Thirteen days and 1 year later, quisqualate-induced septal lesions were associated with a substantial disappearance of cholinergic nerve cell bodies and their nerve terminal networks within the hippocampal formation, the remaining cholinergic nerve terminals having significantly increased specific mean grey values as studied by acetylcholine esterase immunohistochemistry in combination with image analysis in the dentate gyrus. In contrast, ibotenic acid injections into the medial septal nucleus did not result in any noticeable disappearance of hippocampal cholinergic nerve terminals at the 13 day time point and at the 1 year interval only a nonsignificant reduction was obtained in the dentate gyrus. Within the ventral part of globus pallidus, but not within the medial septal nucleus, the nerve cell death was associated with the appearance of calcium deposits within the large pallidal neurons. This phenomenon began already at the early time interval and appeared maximal at the 1 year interval. Thus, at this time large calcium deposits (ranging from 500 to 1600 μm^2 in size) were found extracellularly as rounded profiles (≈100-300 per side and section) throughout the entire ventral globus pallidus. One year after septal lesions induced by either ibotenic acid or quisqualate a marked atrophy of the entire dorsolateral septal nucleus was observed, together with a marked increase in Fos-IR in the ventrolateral septum, possibly due to an ongoing neurotoxic process different from the one observed within the globus pallidus.

Thus, long term excitotoxic lesions of these two brain areas resulted in marked differences in neuronal damage that may be related to a differential distribution of the glutamate subtype receptors. Also, the present findings give evidence that calcium toxicity may be a major factor in the mediation of excitotoxin induced nerve cell death within globus pallidus.

ALZHEIMER ß-AMYLOID PRECURSOR PROTEIN PROCESSING IN C6 GLIOMA CELLS

F.Mayor,jr.and E.Morato
Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM). Universidad Autonoma.28049 Madrid .Spain

Altered expression or proteolytic processing of the ß-amyloid protein precursor (ßAPP) seems to play a key role in the pathogenesis of Alzheimer disease (Selkoe(1991) Neuron 6:487-498).Normal processing of ßAPP involves a cleavage by a "secretase" in the ß-peptide region, releasing a large soluble extramembranous portion and retaining a 10K C-terminal fragment in the membrane, which is non-amyloidogenic . Recent evidence indicate that alternative cleavage of ßAPP, probably in the endosomal-lysosomal compartment, can generate a variety of carboxyl-terminal fragments of ßAPP, that contain the entire ß/A4 sequence, and that may eventually lead to amyloid deposition and neurotoxicity (Estus et al . Science 255, 726-730, 1992). Moreover,recent reports have put forward the unexpected finding that a soluble form of the ß/A4 peptide is produced by cells during normal metabolism (Haass et al. (1992) Nature 359:322-325;Shoji et al. (1992)Science 258:126-129).In this context, we have investigated the subcellular location and proteolytic processing of ßAPP in an experimental model of glial cells in culture (C6 glioma cells), by using several affinity-purified antipeptide antibodies raised in our laboratory against different domains of both the ß/A4 peptide and ßAPP.

Our data indicate the existence of a high expression and active metabolism of ßAPP in glial cells .Moreover,immunofluorescence studies clearly show a preferential intracellular location for ßAPP antigens and the accumulation of reinternalized ßAPP in endosomes/lysosomes in the presence of inhibitors of lysosomal proteases.Finally,we have detected with several antibodies raised against the N and C- terminal regions of the ß/A4 peptide the presence of a soluble 4 Kda peptide in the conditioned medium of C6 glioma cells,in line with the above mentioned reports in other cell types.Given the abundance of glial cells in the nervous tissue, our results pose important questions regarding the physiological role of the ß/A4 peptide and its precursor fragments ,the cellular pathways involved in their generation and its possible alteration in pathological circumstances.

Supported by CAM C105/91,Boehringer Ingelheim and Fundación Ramon Areces

DIFFERENTIAL LABELLING OF A SUBPOPULATION OF PLAQUES WITH MAB 423 AND FLUORESCENT MARKER FOR INTRACELLULAR TANGLES.

Authors: Mena R.[1], Edwards P.[2], Wischik CM[2].

Dept. of Physiology and Neurosciences[1], CINVESTAV-IPN, P.O. Box 14-740, 07000, México, D.F. México, Cambridge Brain Bank Laboratory[2], Department of Psychiatry, University of Cambridge Clinical School, Hills Rd., Cambridge CB2 2QQ, U. K.

The monoclonal antibody (mAb) 6.423 recognizes a specific truncation at Glu391 of the paired helical filament tau protein in Alzheimer's disease (Novak et al., EMBO J. 12:365,1993). A previous study has shown that mAb 6.423 stains intracellular neurofibrillary tangles (NFT's) in AD cases with short dementia duration, and extracellular NFT's and "ghost neurites" in cases of longer clinical duration. Strikingly, plaque-like structures possesing a granular appearance were also identified in the latter cases (Mena et al., J. Neuropathol. Exp. Neurol.50:474,1991). This observation could be explained by the presence of PHFs deriving from extracellular tangles or neurites which have undergone further degradation in the extracellular space. In the present study we used a laser-scanning confocal microscope to investigate the relationship between mAb 6.423 immunoreactivity and labelling by the fluorescent dye TR 450 in these mAb 6.423-immunoreactive plaques. TR 450 has staining properties similar to Thioflavin S, except that it is a chemically purer preparation, and fluoresces red (Wischik CM. PhD Thesis, University of Cambridge, 1989; Resch et al. Biorganic Med. Chem. Lett. 1 (10):519,1991). In particular, TR 450 labels intra-and extracelullar tangles, as well as dystrophic neurites in the neuropil, as well as labelling amyloid plaque cores. We found that the mAb 6.423-immunoreactive plaques were not labelled with TR 450. mAb 643-immunoreactive neuritic clumps and granular staining were observed in regions where TR 450-labelled plaques were absent. Likewise, 6.423-reactive structures were often sparse in regions where TR 450-labelled plaques could be found. We interpret these findings as suggesting that the granular mAb-6.423 immunoreactivity seen in some plaques is due to further degradation of extracellular PHFs deriving from ghost tangles and ghost neurites. Interestingly, this presumably proteolytic degradation appears to occur without loss of mAb 6.423 immunoreactivity, but loss of the TR 450 binding which is seen in intracellular tangles. These findings suggest that the TR 450 and mAb 6.423 binding sites are distinct. **Partially supported (R.M.) by a COSNET-SEP grant 147.92-E.**

TAU TRANSFECTION IN COS-1 CELLS STABILIZES CENTROSOME-INDEPENDENT MICROTUBULES AND PROMOTES THE EXTENSION OF CYTOPLASMIC PROCESSES.

E. Montejo de Garcini, S. de la Luna and M. Medina.

Centro de Biología Molecular and Centro Nacional de Biotecnología. Cantoblanco. Madrid. Spain.

COS-1 cells were transfected with cDNAs coding for different human tau isoforms, which results in a high level of tau protein expression. Expressed tau isoforms bind to cellular microtubules *in vivo*, preferentially at the distal regions of microtubules nucleated by the centrosome, leading to their stabilization. Eventually, tau-coated microtubules without any association with the centrosome were observed. Interestingly, all transfected tau isoforms were found to be phosphorylated *in vivo* and a certain phosphorylation level of all tau proteins is required to allow their binding to cellular microtubules. A major difference between tau isoforms containing three tubulin-binding motifs and tau isoforms containing four tubulin-binding motifs is the greater ability of the latter in inducing the formation of long cytoplasmic processes.

ASTROCYTES, NORMAL AGING AND ALZHEIMER DISEASE

F. Mora and M. Nieto-Sampedro
Department of Physiology. Faculty of Medicine
University Complutense of Madrid
28040 Madrid (Spain)
and
Neural Plasticity Group
Instituto Cajal (CSIC) Madrid (Spain)

Increasing evidence has accumulated indicating a major role for astrocytes in the compesatory mechanisms activated in the brain as response to neuron degeneration during aging. Thus, astrocytes increase in number during the normal aging process (3,4) and show an increase in metabolic precursors for the synthesis of neurotransmitters in specific areas of the brain (1). This, together with the physiological role of astrocytes in the removal from the extracellular space of excess exitatory molecules and iones, the detoxification of deleterious substances, such as ammonium, the production of immune mediators and the production of neurotrophic and neurite factors, make astrocytes the cells which could be the primary controllers of the CNS homeostasis and plasticity during the normal aging process (2).

Recent evidence has implicated astrocytes in the pathogenesis of Alzheimer disease. We have proposed recently that amyloid precursor protein (APP) overexpression in reactive astrocytes, as a result of vascular problems or neuron atrophy, could trigger a cascade of events, beginning with cytotoxic intracellular accumulation of APP fragments and astrocyte swelling (5). The resulting impairment in local homeostasis and the extracellular accumulation of excitatory neurotransmitters, could reach excitotoxic levels followed by neural death and further gliosis. Recent clinical reports give support to these ideas (2).

Supported by DGICYT PB90-0252 and CICYT/SAP92-0212

1) Cobo, M. Exposito, I., Porras, A., Mora, F. Neurobiol. Aging 13, 705-709 (1992).
2) Nieto-Sampedro, M., Mora F., TINS 1993 (submitted)
3) Terry, R.D., Prog. Brain Res. 70, 41-48 (1986)
4) Vazquez, M.T., Prados, J.C., Puerta, A.J., Mora, F. Europ. J. Neurosci, Suppl. 5, 100 (1992)
5) Yoshikawa, K. Nature 361, 122-123 (1993)

LOOKING FOR A NOVEL LOCUS FOR FAMILIAL ALZHEIMER'S DISEASE.

Marzia Mortilla, Sandro Sorbi, Luigi Amaducci, *Peter St.George-Hyslop.
Dept. of Neurology, University of Florence, Florence, Italy.
*CRND, University of Toronto, Toronto, Ontario, Canada.

In the past few years several linkage studies lead to the hypothesis of a genetic heterogeneity in Familial Alzheimer's disease (FAD). Some families showed a lod score above 2 with markers from the proximal long arm of chromosome 21; for other families, with the late onset form, a locus on chromosome 19 has been suggested; another small proportion of early onset pedigrees turned to be associated with mutations in the amyloid precursor protein (APP) gene on chromosome 21. Since the majority of the families did not showed linkage with those two chromosomes we started a screening of our pedigrees with Simple Sequence Repeats (SSR) markers from chromosomes other than 19 and 21.
In a series of 21 pedigrees we provided evidence for a linkage (multipoint lod score z= 23.4) with some markers from the long arm of chromosome 14.
Three markers (D14S53, D14S43, D14S55) spanning a 12cM region in the center of the long arm of chromosome 14 gave highly significant two point lod score in the overall group but the more interesting results are evident looking at the families singularly. Six independent pedigrees provided statistically significant lod scores for at least one of these markers.
Our present studies are following three directions: 1) Analysis of all the other Italian pedigrees with the markers D14S53, D14S43, D14S55; 2) Analysis of the pedigrees that don't show linkage with the chromosomes 21, 19, 14 with markers from different chromosomes; 3) Analysis of the chr.14-linkad pedigrees in the 12cM region described above, infact in the vicinity of the markers that give positive lod scores some candidate genes are located. Among those gene alfa-1-anti-chymotrypsin and the protease Cathepsin G are already excluded to be the FAD locus because of the negative results obtained with linkage. We are investigating other genes such as the FBJ osteosarcoma viral oncogene homologue (cFOS) and the heat shock proteins (HSP70 and HPS90) in order to establish whether they might be the site of an FAD mutation in our pedigrees.

Truncation of tau in Alzheimer's disease

Novak,M.[1,2] Cuello,A.C.[3] Milstein,C.[1]
[1] MRC, Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.
[2] Institute of Virology, SAS, 842 46 Bratislava, Slovakia
[3]McGill University,Montreal,Canada

Abnormal sequestration of microtubule associated protein tau in the form of paired helical filaments (PHFs) is hallmark of Alzheimer's disease. Its abnormal phosphorylation in the course of the disease is now well documented. We have prepared two PHF tau specific phosphorylation independent monoclonal antibodies (6.423 and 7.28). Monoclonal antibody 423 decorates PHF and recognizes a 12 kDa tau fragment released from the core PHF, but does not react with full length native or expressed tau. C-terminus of 12 kDa fragment was crucial for mAb 423 immunoreactivity. Molecular expression of the fragment showed that mAb 423 recognizes all and only those PHF core tau analogues which terminate at Glu 391. Addition or removal of a single residue at the C-terminus abolishes immunoreactivity. Therefore mAb 423 is a marker for both exogenous and endogenous truncation of tau at Glu 391 and together with the knowledge of the N-terminus can be used to measure the extent of 12 kDa PHF core fragment. This we term the minimal protease resistant tau unit of the core PHF. Histological localisation of this truncation shows that 423 epitope is present in intracellular granular and neurofibrillary degeneration in Alzheimer's disease tissues. Monoclonal antibody 7.28 reacts with 12 kDa fragment but does not compete with mAb 423 for binding site on this PHF core tau fragment. The antibody, like mAb423, does not recognise normal full length tau. cDNA synthesis and expression of candidate minimal protease resistant tau units permitted the mapping of the 7.28 epitope. mAb 7.28 recognizes all and only those candidate peptides which have a similar size and the same sequence as genuine minimal protease resistant tau units of the PHF core. Extension or reduction in length either at the N- or at the C-terminus abolishes mAb 7.28 immunoreactivity. Similarly to mAb 423, mAb 7.28 stains in Alzheimer's disease tissues dystrophic neurites and neurofibrillary tangles suggesting that mAb 7.28 can be used for identification of an unique conformation of N- and C-terminally truncated tau in Alzheimer's disease.

References

Goedert,M. and Jakes,R. (1990) *EMBO J.*, **9**, 4225-4230.
Goedert,M., Spillantini,M.G., Cairns,N.J. and Crowther,R.A.(1992), *Neuron* **8**, 159-168.
Jakes,R., Novak,M., Davison,M. and Wischik,C.M. (1991) *EMBO J.*, **10**, 2725-2729.
Mena,R., Wischik,C.M., Novak,M., Milstein,C. and Cuello C.A. (1991) *J.Neuropathol. Exp.Neurol.*, **50**, 474-490.
Novak,M., Jakes,R., Edwards,P., Milstein,C. & Wischik.C.(1991) *Proc.Natl.Sci.USA*, **88**, 5837-5841.
Novak,M., Wischik,C.M., Edwards,P.C., Pannell,R. and Milstein,C. (1989) *Progr. Clin. Biol. Res.* **317**, 755-761.
Novak,M., Kabat,J and Wischik,C. (1993), *EMBO J.*,**12**, 365-370.
Wischik, C.M., Novak, M., Thogersen,H.C., Edwards,P.C., Runswick,M.J., Jakes,R., Walker,J.E., Milstein,C., Roth,M and Klug,A. (1988a) *Proc. Natl. Acad. Sci.* USA 85, 4506-4510.

APP VARIANTS IN SPANISH ALZHEIMER AND CONTROL CASES

Adroer, R. and Oliva, R.

Grupo de Genetica Molecular, Faculty of Medicine, University of Barcelona, Diagonal 643, 08028 Barcelona, Spain. FAX: 34 3 4909346.

Since APP mutations are infrequent (Goate et al., 1991, Nature 349, 704-706) and a large number of samples must be sequenced in order to identify this or other mutations in different populations, we optimized the procedure for sample processing before sequencing reducing the time from 11 h to 3,5 h (Adroer, R., Chartier-Harlin, M.C., Crawford, F. and Oliva, R. (1992). Improved direct sequencing of Alzheimer's amyloid precursor protein exons 16 and 17. Neuroscience Letters 141, 69-71). Using this method to sequence exons 16 and 17 following SSCA analysis of Spanish Alzheimer and control cases revealed a novel mutation at codon 711 and a silent variant at codon 708 in patients belonging to the Alzheimer Families Association from Catalunya (AFA) (Adroer, R., López-Acedo, C., Oliva, R., Hardy, J. and Fidani, L. (1992). A normal silent variant at codon 711 and a variant at codon 708 of the APP sequence detected in Spanish Alzheimer and control cases. Neuroscience Letters, in press). Further screening of 34 additional Alzheimer patients from local hospitals and AFA revealed normal sequences in all of them. Thus the overall frequency of the reported APP mutations is 1.7% of the screened Alzheimer families for the APP717 Val to Ile (pathogenic), 1.4% for APP711 (silent at the protein level, but not found after sequencing 100 unrelated control cases) and 1.3% for APP708 present in control cases as well as Alzheimer patients (silent and non pathogenic). We are planning to extent the search for APP variants with 100 additional Alzheimer patients. In addition to the detection of new or described APP mutations, we are also documenting these families with a view to search for potential mutations present in other loci.

Supported by a grant form CICYT (SAL90-0334) to R.O.

Calcium regulation in fibroblasts derived from skin biopsies with varying amyloid β-protein deposition. C. Börner[1], R. Pereira[1], A. Martínez-Serrano[1], M.Villalba[1], C. Hernández[1], B. Merinero[1], C. Perez-Cerdá[1], E. Bogónez[1], M. Ugarte[1], A. Cuevas[5], A. Marqués[2], F. Coria[4], J. C. Alvarez-Cermeño[2], L.M. García-Segura[3] and J. Satrústegui[1]. [1]Departamento de Biología Molecular. Centro de Biología Molecular "Severo Ochoa". C.S.I.C.-Univ. Autónoma de Madrid. 28049-Madrid [2]Servicios de Neurología y Dermatología. Hospital Ramón y Cajal. 28034-Madrid. [3]Instituo Cajal. C.S.I.C. 28002-Madrid. [4]Departamento de Neurología. Hospital General. 40002-Segovia , [5]Departamento de Matemáticas, Facultad de Ciencias, Universidad Autónoma de Madrid, 28049-Madrid. SPAIN.

Amyloid β-protein (βAP) deposits are found in tissues other than brain in individuals with Alzheimer's disease (AD) and normal ageing (Joachim et al, Nature 241 (1989) 226-230). The relation of these deposits with the function of the neighbouring cells is still unknown. Human fibroblasts from AD patients have been reported to have lower cytosolic calcium levels and cell spreading when compared to age-matched controls (Peterson et al. Proc. Natl. Acad. Sci. USA 83 (1986) 7999-8001). The expression of the βAP precursor by human fibroblasts increases during in vitro ageing (Adler et al. Proc. Natl. Acad. Sci. USA 88 (1991) 16-20). The purpose of this work was to study a possible causal relationship between βAP deposition in skin biopsies and changes in cytosolic calcium regulation in fibroblasts derived from them. Human skin biopsies were obtained form 12 AD patients and 12 age-matched controls (age range 43-80 yrs). βAP deposition was evaluated by immunostaining with a rabbit polyclonal antiserum raised against the native, HPLC-purified βAP prepared from amyloid-rich filament fractions of AD cerebral cortex (Selkoe et al. J. Neurochem. 46 (1986) 1820-1834) which was kindly supplied by D. Selkoe. All of the AD and 5 control biopsies examined were βAP immunoreactive (with varying reactivities), in agreement with previous reports. Cytosolic calcium determinations were carried out in the corresponding fibroblasts (after 6-21 population doublings) with fura2 microfluorimetry. Fura2 staining was uniform ruling out a possible compartmentalization of the dye. Resting cytosolic calcium levels decreased with ageing and in AD patients. This appeared to be associated with an increased strength of calcium buffering of external calcium loads as mediated by long (60 min) incubation with the calcium ionophore BrA23187 (1 μM). We have not found any clear relation between cytosolic calcium levels and βAP deposition, except for a significant increase in intracellular calcium stores (as evaluated by peak $[Ca^{2+}]_i$ after 1μM BrA23187 addition) in those individuals (controls or AD) with mild bAP immunoreactivity. Studies are now underway to analyze whether the size of the intracellular calcium stores may be involved in the production/processing of βAP precursor by skin-derived fibroblasts.

Supported by Boehringer Ingelheim España S.A. and by the Fundación Ramón Areces.

THE ROLE OF βAMYLOID PEPTIDE AS A DIAGNOSTIC AND PROGNOSTIC MARKER OF ALZHEIMER'S DISEASE

C. Vigo-Pelfrey

Athena Neurosciences Inc., 800F Gateway Boulevard
South San Francisco, CA. 94080 (USA)

Cerebral deposition of the β-amyloid peptide (Aβ) is an invariant feature of Alzheimer's disease (AD). Aβ has been isolated from extracellular spaces of the brain and the cerebral blood vessels of patients with AD, trisonomy 21, hereditary cerebral hemorrhage with amyloidosis -- Dutch type and undiseased aged brain. An extensive search has been conducted for evidence that Aβ is produced and released in bodily fluids *in vivo* and *in vitro*. Until recently, direct attempts to demonstrate the presence of Aβ in human plasma or cerebrospinal fluid (CSF) have been unsuccessful.

Using monoclonal antibodies specific for an epitope within Aβ that spans the size of normal constitutive cleavage, we have recently demonstrated the presence of Aβ in plasma, CSF and conditioned media of various cell cultures.

Using affinity chromatography we have purified and sequenced Aβ and related fragments from CSF and conditioned media of human mixed brain cell cultures. Laser desorption mass spectrometry studies indicated the presence of various Aβ species in CSF containing 1-27, 1-28, 1-30, 1-35, 1-38, 1-40, 12-43, 6-27, 6-34, 6-35, 3-34, 11-43 amino acids and various stable Aβ dimers and trimers.

These findings show that Aβ is present as a soluble molecule in CSF, despite this peptide's original characterization as a highly unsoluble substance. Chronically enhanced production or decrease in clearance of Aβ in CSF might result in gradual formation of aggregates and finally deposition in AD senile plaques.

We have also studied the diagnostic significance of the presence of Aβ in CSF. In a preliminary study using CSF from 100 AD patients and matched controls, we have showed that Aβ is elevated in AD patients, thus resulting in a very valuable marker for the diagnosis and therapeutic strategies targeted to the modulation of Aβ production in AD.

Furthermore, with the demonstration of Aβ production by various cells in culture, untransfected or transfected with constructs containing the mutation characteristic of various familial AD, we have established a model to study *in vitro* the modulation of Aβ release and its inhibition by drugs.

DIFFERENCES ON ß-AMYLOID PRECURSOR PROTEIN GLYCOSYLATION AFTER BRAIN DAMAGE

Olga Salinero , Maria Araujo , JuanJose Garrido and Francisco Wandosell.
Neurodegeneration Group. Avd. Dr. ARCE 37 CSIC Madrid 28002 Spain

Extracellular deposition of ß4A amyloid peptide is the most important feature of Alzheimer disease, and could be the base of neuronal degeneration. The ß4A is derived from a family of membrane glycoproteins, termed amyloid precursor proteins (APP). The extracellular portion of some APP isoforms contain a region with high homology to Kunitz-type serin-protease inhibitor.After glycosylation APP's are cleaved at the extracellular portion and secreted.Two different mechanisms of cleavage have been postulated, and one of these produce the amiloidogenic fragment of 4K. Once this fragment is produced, it undermines the viability of neurons by a mechanisms not well understood.

We wish to analyze, the effect of some brain damages in the glycosylation pattern of APP's, and how after brain injury ,it can change these modifications and the APP proteolitic pattern. For that purpose, we have developped a series of polyclonal antibodies against synthetic peptides of APP.

First, we have studied the expression of APP after controled hippocampal damage, by intraventricular injection of glutamate analogs. The glutamate receptor types have been characterized by their selective sensitivity to different agonist (NMDA, Kainic Acid, Quisqualic Acid and APB). We have used some agonist against each type of receptor to produce experimentally induced neuronal damage and after that we have analyzed the expression of APP's. We have used the antibody against a region contained on ß4A,in order to follow the time-course of the damage-induced APP expression.The number and intensity of the labelled cells were different depending on the glutamate analogs used. The co-localization , shows that main cell population containing APP are those double-labelled for GFAP and a minor population double-labelled for OX42.

Second, we have also analyzed the glycosylation state of the APP after those experimental damages and compared with undamage brains. In that sense, APP have been reported to be a secreted proteoglycan, chondroitin sulfate type, in C6 glioma.We have purified the proteoglycan fraction from brain and we have analyzed the fraction of those being APP's

The APP's containing the sequence homologous to Kunit-type serine protease inhibitor were selected by affinity chromatography, from total and membrane fractions after kainate-injured brains. This fraction of APP's were treated with several glycosidases, and then analyzed by Western-blots with some antibodies against different regions of APP. Our data show clearly two proteoglycan populations and differences between damaged and undamaged brains.

Supported by grants from CAM192/90 and DGYCT PB90-0160.
O.S. is supported by a fellowship from M.E.C.

SUMMARY

J. Avila

There are two clasical features of Alzheimer's disease (AD), plaques and tangles, described more than 80 years ago by Dr. Alzheimer. Plaques and tangles are aberrant brain structures which molecular composition has been very recently indicated. The meeting, held at Fundation March, has been mainly focus in the characteristics of components for those structures; their expresion, their processing and their folding. By taking into account those steps has been discussed the assembly and formation of amyloid deposits, components of the plaques; and that of paired helical filaments, components of tangles. Additionally, it has been discussed other molecules which can interact with the previous ones and that there related with some genetic disorders related to Alzheimer's disease.

At the first part of the meeting the role of tau, a microtubule associated protein, in building paired helical filaments (probably after being modified by phosphorylation), was discussed.

In the second part of the meeting the way in which Aß builts amyloid deposites was presented. Aß is a fragment of a larger protein , APP, and the cleavage mechanisms of the latter was a matter of discussion. Also it was discussed if APP has a neural or a non neural origen.

At the last part of the meeting the use of an animal model, to study the disease, and the way of how to appley the current knowledge was mainly the subject. Unfortunately, a positive answer for Alzheimer's disease therapy was not given at the present.

List of Invited Speakers

Workshop on

FRONTIERS OF ALZHEIMER DISEASE

List of Invited Speakers

J. Avila	Centro de Biología Molecular , (CSIC-UAM), Facultad de Ciencias, Universidad Autónoma, Cantoblanco, 28049 Madrid (Spain). Tel.: 34 1 397 84 40 Fax : 34 1 397 47 99
K. Beyreuther	Center for Molecular Biology Heidelberg, University of Heidelberg, Im Neuenheimer Feld 282, D-6900 Heidelberg (Germany). Fax : 49 6221 56 58 91
D.D. Cunningham	Department of Microbiology & Molecular Genetics, University of California, College of Medicine, Irvine, CA. 92717-4025 (USA). Tel.: 1 714 856 70 74 Fax : 1 714 856 85 98
A. Delacourte	Unité 156 INSERM, Place de Verdun, 59045 Lille (France). Tel.: 33 20 53 43 03 Fax : 33 20 52 37 94
B. Frangione	Department of Pathology, New York University Medical Center, 550 First Avenue, New York, NY. 10016 (USA). Tel.: 1 212 263 57 75 Fax : 1 212 263 67 51
C. Gajdusek	Department of Health & Human Services, National Institutes of Health, NINDS, Laboratory of Central Nervous System Studies, Bethesda, MD. 20892 (USA). Tel.: 1 301 496 32 81 Fax : 1 301 496 82 75
M. Goedert	MRC - Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH (U.K.). Tel.: 44 223 248 011 Fax : 44 223 213 556

Y. Ihara	Department of Neuropathology, Institute of Brain Research, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-Ku, Tokyo 113 (Japan). Tel.: 81 3 3812 2111 Ext. 3541 Fax : 81 3 5800 6852
K. Iqbal	Chemical Neuropathology Laboratory, Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY. 10314-6399 (USA). Tel.: 1 718 494 52 59 Fax : 1 718 494 52 69
K.S. Kosik	Harvard Medical School and Center for Neurologic Diseases, Department of Medicine (Division of Neurology), Brigham and Women's Hospital, Boston, MA. 02115 (USA). Tel.: 1 617 732 64 60 Fax : 1 617 732 77 87
C. Milstein	MRC - Laboratory of Molecular Biology, Protein and Nucleic Acid Chemistry Division, Hills Road, Cambridge CB2 2QH (U.K.). Tel.: 44 223 24 80 11 Fax : 44 223 41 21 78
D.L. Price	Johns Hopkins University, Neuropathology Laboratory, School of Medicine, 720 Rutland Avenue, 558 Ross Research Building, Baltimore, MD. 21205-2196 (USA). Tel.: 1 410 955 56 32 Fax : 1 401 955 97 77
A. Probst	Institut für Pathologie, Kantonsspital Basel Universitätskliniken, Schönbeinstraße 40, CH-4003 Basel (Switzerland) Tel.: 41 61 265 27 57 Fax : 41 61 261 30 22
N. K. Robakis	The Mount Sinai Medical Center, Department of Psychiatry & Neurobiology Center, One Gustave L. Levy Place, Box 1229, New York, NY. 10029-6574 (USA). Tel.: 1 212 241 93 80 Fax . 1 212 831 19 47

A. D. Roses — Joseph and Kathleen Bryan Alzheimer's Disease Center, Division of Neurology, Duke University Medical Center, Durham, NC. 27710 (USA).
Tel.: 1 919 684 62 74
Fax : 1 919 684 65 14

S.S. Sisodia — Neuropathology Laboratory, The Johns Hopkins University, School of Medicine, 720 Rutland Avenue, 558 Ross Research Building, Baltimore. MD. 21205-2196 (USA).
Tel.: 1 410 955 56 32
Fax : 1 410 955 97 77

C.J. Smith — Department of Neuroscience, Institute of Psichiatry, London SE5 8AF (U.K.).
Tel.: 44 71 703 54 11
Fax : 44 71 277 13 90

W.G. Turnell — MRC - Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH (U.K.).
Tel.: 44 223 24 80 11
Fax : 44 223 21 35 56

H.M. Wisniewski — Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY. 10314-6399 (USA)
Tel.: 1 718 494 06 00
Fax : 1 718 698 38 03

List of Participants

Workshop on

FRONTIERS OF ALZHEIMER DISEASE

List of Participants

K.C. Breen — Department of Pharmacology & Clinical Pharmacology, Ninewells Hospital & Medical School, University of Dundee, Dundee DD1 9SY, Scotland (U.K.).
Tel.L 44 382 60 111 Ext. 2522
Fax : 44 382 67 120

F. Collia — Departamento de Anatomía e Histología Humanas, Facultad de Medicina, Universidad de Salamanca, Avda. del Campo Charro, s/nº, 37007 Salamanca (Spain).
Tel.: 34 23 29 45 44
Fax : 34 23 29 45 10

F. Coria — Departamento de Neurología, Laboratorio de Investigaciones Neurológicas, Complejo Hospitalario de Segovia, Ctra. de Ávila, s/nº, 40002 Segovia (Spain).
Tel.: 34 11 41 90 69
Fax : 34 11 44 22 35

A. Chauhan — Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY. 10314-6399 (USA).
Tel.: 1 718 494 06 00
Fax . 1 718 698 38 03

D.C. Davies — Department of Anatomy, St. George's Hospital Medical School, Cranmer Terrace, Tooting, London SW17 0RE (U.K.)
Tel.: 44 81 672 99 44 Ext. 55211
Fax : 44 81 767 46 96

P. Gómez-Ramos — Departamento de Morfología, Facultad de Medicina, Universidad Autónoma, Arzobispo Morcillo, s/nº, 28029 Madrid (Spain).
Tel.: 34 1 397 84 40
Fax : 34 1 397 47 99

N. Mahy — Departamento de Ciencias Fisiológicas, Unidad de Bioquímica, Facultad de Medicina, Universidad de Barcelona, Avda. Diagonal, 643, 08028 Barcelona (Spain).
Tel.: 34 3 339 71 86
Fax . 34 3 490 93 46

F. Mayor — Centro de Biología Molecular, (CSIC-UAM), Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid (Spain).
Tel.: 34 1 397 50 70
Fax : 34 1 397 47 99

M. Medina — Centro de Biología Molecular, (CSIC-UAM), Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid (Spain).
Tel.: 34 1 397 50 70
Fax : 34 1 397 47 99

R. Mena — Departamento de Fisiología, Biofísica y Neurociencias, Centro de Investigación y de Estudios Avanzados del I.P.N., P.O. Box 14-740 07000 México, D.F. (Mexico).
Tel.: 525 754 02 00
Fax : 525 586 65 64

E. Montejo de Garcini — Centro de Biología Molecular, (CSIC-UAM),
Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid (Spain).
Tel.: 34 1 397 50 70
Fax : 34 1 397 47 99

F. Mora — Departamento de Fisiología, Facultad de Medicina, Universidad Complutense de Madrid, Ciudad Universitaria, 28040 Madrid (Spain).
Fax : 34 1 394 16 28

A. Morán — Departamento de Morfología, Facultad de Medicina, Universidad Autónoma de Madrid, Avda. Arzobispo Morcillo, s/nº, 28029 Madrid (Spain).
Tel.: 34 1 397 53 60
Fax : 34 1 315 00 75

E. Morato — Departamento de Biología Molecular, Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid (Spain).
Fax : 34 1 397 48 70

M. Mortilla — Department of Neurology, University of Florence, Viale Morgagni, 85, 50134 Florence (Italy).
Tel.: 39 55 422 27 67
Fax : 39 55 418 71 8

M. Nieto-Sampedro — Instituto Cajal, Avda. Dr. Arce, 37, 28002 Madrid (Spain).
Fax : 34 1 585 47 54

M. Novak	MRC - Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH (U.K.). Tel.: 44 223 40 23 90 Fax : 44 223 41 22 82
R. Oliva	Grupo de Genética Molecular, Unidad de Fisiología, Facultad de Medicina, Universidad de Barcelona, Diagonal 643, 08028 Barcelona (Spain). Tel.: 34 3 339 78 85 Fax : 34 3 490 93 46
D. Pulido	Centro de Biología Molecular, (CSIC-UAM), Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid (Spain). Tel.: 34 1 397 50 72 Fax : 34 1 397 47 99
S.R. Sahasrabudhe	Department of CNS Biology, Lederle Laboratories, 277 Hobart Street, Pearl River, NY. 10965 (USA). Tel.: 1 914 732 30 88 Fax : 1 908 231 43 23
J. Satrústegui	Centro de Biología Molecular, (CSIC-UAM), Facultad de Ciencias, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid (Spain). Tel.: 34 1 397 50 72 Fax : 34 1 397 47 99
M.G. Spillantini	MRC - Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH (U.K.). Tel.: 44 223 40 23 90 Fax : 44 223 41 22 82
C. Vigo-Pelfrey	Athena Neurosciences Inc., 800F Gateway Boulevard, South San Francisco, CA. 94080 (USA). Tel.: 1 415 877 09 00 Fax : 1 415 877 83 70
F. Wandosell	Instituto Cajal, C.S.I.C., Avda. Dr. Arce, 37, 28002 Madrid (Spain). Tel.: 34 1 585 41 50 Fax : 34 1 585 47 54

Texts published in the
SERIE UNIVERSITARIA
by the
FUNDACIÓN JUAN MARCH
concerning workshops and courses organized within the
Plan for International Meetings on Biology (1989-1991)

246 **Workshop on Tolerance: Mechanisms and implications.**
Organized by P. Marrack and C. Martínez-A. Lectures by H. von Boehmer, J. W. Kappler, C. Martínez-A., H. Waldmann, N. Le Douarin, J. Sprent, P. Matzinger, R. H. Schwartz, M. Weigert, A. Coutinho, C. C. Goodnow, A. L. DeFranco and P. Marrack.

247 **Workshop on Pathogenesis-related Proteins in Plants.**
Organized by V. Conejero and L. C. Van Loon. Lectures by L. C. Van Loon, R. Fraser, J. F. Antoniw, M. Legrand, Y. Ohashi, F. Meins, T. Boller, V. Conejero, C. A. Ryan, D. F. Klessig, J. F. Bol, A. Leyva and F. García-Olmedo.

248 Beato, M.:
Course on DNA - Protein Interaction.

249 **Workshop on Molecular Diagnosis of Cancer.**
Organized by M. Perucho and P. García Barreno. Lectures by F. McCormick, A. Pellicer, J. L. Bos, M. Perucho, R. A. Weinberg, E. Harlow, E. R. Fearon, M. Schwab, F. W. Alt, R. Dalla Favera, P. E. Reddy, E. M. de Villiers, D. Slamon, I. B. Roninson, J. Groffen and M. Barbacid.

251 **Lecture Course on Approaches to Plant Development.**
Organized by P. Puigdoménech and T. Nelson. Lectures by I. Sussex, R. S. Poethig, M. Delseny, M. Freeling, S. C. de Vries, J. H. Rothman, J. Modolell, F. Salamini, M. A. Estelle, J. M. Martínez Zapater, A. Spena, P. J. J. Hooykaas, T. Nelson, P. Puigdoménech and M. Pagès.

252 **Curso Experimental de Electroforesis Bidimensional de Alta Resolución.**
Organizado por Juan F. Santarén. Seminarios por Julio E. Celis, James I. Garrels, Joël Vandekerckhove, Juan F. Santarén y Rosa Assiego.

253 **Workshop on Genome Expression and Pathogenesis of Plant RNA Viruses.**
Organized by F. García-Arenal and P. Palukaitis. Lectures by D. Baulcome, R. N. Beachy, G. Boccardo, J. Bol, G. Bruening, J. Burgyan, J. R. Díaz Ruiz, W. G. Dougherty, F. García-Arenal, W. L. Gerlach, A. L. Haenni, E. M. J. Jaspars, D. L. Nuss, P. Palukaitis, Y. Watanabe and M. Zaitlin.

254 **Advanced Course on Biochemistry and Genetics of Yeast.**
Organized by C. Gancedo, J. M. Gancedo, M. A. Delgado and I. L Calderón.

255 **Workshop on The Reference Points in Evolution.**
Organized by P. Alberch and G. A. Dover. Lectures by P. Alberch, P. Bateson, R. J. Britten, B. C. Clarke, S. Conway Morris, G. A. Dover, G. M. Edelman, R. Flavell, A. Fontdevila, A. García-Bellido, G. L. G. Miklos, C. Milstein, A. Moya, G. B. Müller, G. Oster, M. De Renzi, A. Seilacher, S. Stearns, E. S. Vrba, G. P Wagner, D. B. Wake and A. Wilson.

256 **Workshop on Chromatin Structure and Gene Expression.**
Organized by F. Azorín, M. Beato and A. A. Travers. Lectures by F. Azorín, M. Beato, H. Cedar, R. Chalkley, M. E. A. Churchill, D. Clark, C. Crane-Robinson, J. A. Dabán, S. C. R. Elgin, M. Grunstein, G. L. Hager, W. Hörz, T. Koller, U. K. Laemmli, E. Di Mauro, D. Rhodes, T. J. Richmond, A. Ruiz-Carrillo, R. T. Simpson, A. E. Sippel, J. M. Sogo, F. Thoma, A. A. Travers, J. Workman, O. Wrange and C. Wu.

257 **Lecture Course on Polyamines as modulators of Plant Development.**
Organized by A. W. Galston and A. F. Tiburcio. Lectures by N. Bagni, J. A. Creus, E. B. Dumbroff, H. E. Flores, A. W. Galston, J. Martin-Tanguy, D. Serafini-Fracassini, R. D. Slocum, T. A. Smith and A. F. Tiburcio.

258 **Workshop on Flower Development.**
Organized by H. Saedler, J. P. Beltrán and J. Paz Ares. Lectures by P. Albersheim, J. P. Beltrán, E. Coen, G. W. Haughn, J. Leemans, E. Lifschitz, C. Martin, J. M. Martínez-Zapater, E. M. Meyerowitz, J. Paz-Ares, H. Saedler, C. P. Scutt, H. Sommer, R. D. Thompson and K. Tran Thahn Van.

259 **Workshop on Transcription and Replication of Negative Strand RNA Viruses.**
Organized by D. Kolakofsky and J. Ortín. Lectures by A. K. Banerjee, M. A. Billeter, P. Collins, M. T. Franze-Fernández, A. J. Hay, A. Ishihama, D. Kolakofsky, R. M. Krug, J. A. Melero, S. A. Moyer, J. Ortín, P. Palese, R. G. Paterson, A. Portela, M. Schubert, D. F. Summers, N. Tordo and G. W. Wertz.

260 **Lecture Course Molecular Biology of the Rhizobium-Legume Symbiosis.**
Organized by T. Ruiz-Argüeso. Lectures by T. Bisseling, P. Boistard, J. A. Downie, D. W. Emerich, J. Kijne, J. Olivares, T. Ruiz-Argüeso, F. Sánchez and H. P. Spaink.

261 **Workshop The Regulation of Translation in Animal Virus-Infected Cells.**
Organized by N. Sonenberg and L. Carrasco. Lectures by V. Agol, R. Bablanian, L. Carrasco , M. J. Clemens, E. Ehrenfeld, D. Etchison, R. F. Garry, J. W. B. Hershey, A. G. Hovanessian, R. J. Jackson, M. G. Katze, M. B. Mathews, W. C. Merrick, D. J. Rowlands, P. Sarnow, R. J. Schneider, A. J. Shatkin, N. Sonenberg, H. O. Voorma and E. Wimmer.

263 **Lecture Course on the Polymerase Chain Reaction.**
Organized by M. Perucho and E. Martínez-Salas. Lectures by D. Gelfand, K. Hayashi, H. H. Kazazian, E. Martínez-Salas, M. Mc Clelland, K. B. Mullis, C. Oste, M. Perucho and J. Sninsky.

264 **Workshop on Yeast Transport and Energetics.**
Organized by A. Rodríguez-Navarro and R. Lagunas. Lectures by M. R. Chevallier, A. A. Eddy, Y. Eilam, G. F. Fuhrmann, A. Goffeau, M. Höfer, A. Kotyk, D. Kuschmitz, R. Lagunas, C. Leão, L. A. Okorokov, A. Peña, J. Ramos, A. Rodríguez-Navarro, W. A. Scheffers and J. M. Thevelein

265 **Workshop on Adhesion Receptors in the Immune System.**
Organized by T. A. Springer and F. Sánchez-Madrid. Lectures by S. J. Burakoff, A. L. Corbi-López, C. Figdor, B. Furie, J. C. Gutiérrez-Ramos, A. Hamann, N. Hogg, L. Lasky, R. R. Lobb, J. A. López de Castro, B. Malissen, P. Moingeon, K. Okumura, J. C. Paulson, F. Sánchez-Madrid, S. Shaw, T. A. Springer, T. F. Tedder and A. F. Williams.

266 **Workshop on Innovations on Proteases and their Inhibitors: Fundamental and Applied Aspects.**
Organized by F. X. Avilés. Lectures by T. L. Blundell, W. Bode, P. Carbonero, R. W.Carrell, C. S. Craik, T. E. Creighton, E. W. Davie, L. D. Fricker, H. Fritz, R. Huber, J. Kenny, H. Neurath, A. Puigserver, C. A. Ryan, J. J. Sánchez-Serrano, S. Shaltiel, R. L. Stevens, K. Suzuki, V. Turk, J. Vendrell and K. Wüthrich.

267 **Workshop on Role of Glycosyl-Phosphatidylinositol in Cell Signalling.**
Organized by J. M. Mato and J. Larner. Lectures by M. V. Chao, R. V. Farese, J. E. Felíu, G. N. Gaulton, H. U. Häring, C. Jacquemin, J. Larner, M. G. Low, M. Martín Lomas, J. M. Mato, E. Rodríguez-Boulan, G. Romero, G. Rougon, A. R. Saltiel, P. Strålfors and I. Varela-Nieto.

268 **Workshop on Salt Tolerance in Microorganisms and Plants: Physiological and Molecular Aspects.**
Organized by R. Serrano and J. A. Pintor-

Toro. Lectures by L. Adler, E. Blumwald, V. Conejero, W. Epstein, R. F. Gaber, P. M. Hasegawa, C. F. Higgins, C. J. Lamb, A. Läuchli, U. Lüttge, E. Padan, M. Pagès, U. Pick, J. A. Pintor-Toro, R. S. Quatrano, L. Reinhold, A. Rodríguez-Navarro, R. Serrano and R. G. Wyn Jones.

269 **Workshop on Neural Control of Movement in Vertebrates.**
Organized by R. Baker and J. M. Delgado-García. Lectures by C. Acuña, R. Baker, A. H. Bass, A. Berthoz, A. L. Bianchi, J. R. Bloedel, W. Buño, R. E. Burke, R. Caminiti, G. Cheron, J. M. Delgado-García, E. E. Fetz, R. Gallego, S. Grillner, D. Guitton, S. M. Highstein, F. Mora, F. J. Rubia Vila, Y. Shinoda, M. Steriade and P. L. Strick.

Texts published by the CENTRE FOR INTERNATIONAL MEETINGS ON BIOLOGY

1 **Workshop on What do Nociceptors Tell the Brain?**
Organized by C. Belmonte and F. Cerveró. Lectures by C. Belmonte, G. J. Bennet, J. N. Campbell, F. Cerveró, A. W. Duggan, J. Gallar, H. O. Handwerker, M. Koltzenburg, R. H. LaMotte, R. A. Meyer, J. Ochoa, E. R. Perl, H. P. Rang, P. W. Reeh, H. G. Schaible, R. F. Schmidt, J. Szolcsányi, E. Torebjörk and W. D. Willis Jr.

2 **Workshop on DNA Structure and Protein Recognition.**
Organized by A. Klug and J. A. Subirana. Lectures by F. Azorín, D. M. Crothers, R. E. Dickerson, M. D. Frank-Kamenetskii, C. W. Hilbers, R. Kaptein, D. Moras, D. Rhodes, W. Saenger, M. Salas, P. B. Sigler, L. Kohlstaedt, J. A. Subirana, D. Suck, A. Travers and J. C. Wang.

3 **Lecture Course on Palaeobiology: Preparing for the Twenty-First Century.**
Organized by F. Álvarez and S. Conway Morris. Lectures by F. Álvarez, S. Conway Morris, B. Runnegar, A. Seilacher and R. A. Spicer.

4 **Workshop on The Past and the Future of Zea Mays.**
Organized by B. Burr, L. Herrera-Estrella and P. Puigdoménech. Lectures by P. Arruda, J. L. Bennetzen, S. P. Briggs, B. Burr, J. Doebley, H. K. Dooner, M. Fromm, G. Gavazzi, C. Gigot, S. Hake, L. Herrera-Estrella, D. A. Hoisington, J. Kermicle, M. Motto, T. Nelson, G. Neuhaus, P. Puigdomenech, H. Saedler, V. Szabo and A. Viotti.

5 **Workshop on Structure of the Major Histocompatibility complex.**
Organized by A. Arnaiz-Villena and P. Parham. Lectures by A. Arnaiz-Villena, R. E. Bontrop, F. M. Brodsky, R. D. Campbell, E. J. Collins, P. Cresswell, M. Edidin, H. Erlich, L. Flaherty, F. Garrido, R. Germain, T. H. Hansen, G. J. Hämmerling, J. Klein, J. A. López de Castro, A. McMichael, P. Parham, P. Stastny, P. Travers and J. Trowsdale.

6 **Workshop on Behavioural Mechanisms in Evolutionary Perspective.**
Organized by P. Bateson and M. Gomendio. Lectures by J. R. Alberts, G. W. Barlow, P. Bateson, T. R. Birkhead, J. Carranza, C. ten Cate, F. Colmenares, N. B. Davies, R. I. M. Dunbar, J. A. Endler, M. Gomendio, T. Guilford, F. Huntingford, A. Kacelnik, J. Krebs, J. Maynard Smith, A. P. Møller, J. Moreno, G. A. Parker, T. Redondo, D. I. Rubenstein, M. J. Ryan, F. Trillmich and J. C. Wingfield.

7 **Workshop on Transcription Initiation in Prokaryotes.**
Organized by M. Salas and L. B. Rothman-Denes. Lectures by S. Adhya, H. Bujard, S. Busby, M. J. Chamberlin, R. H. Ebright, M. Espinosa, E. P. Geiduschek, R. L. Gourse, C. A. Gross, S. Kustu, J. Roberts, L. B. Rothman-Denes, M. Salas, R. Schleif, P. Stragier and W. C. Suh.

8 **Workshop on the Diversity of the Immunoglobulin Superfamily.**
Organized by A. N. Barclay and J. Vives. Lectures by A. N. Barclay, H. G. Boman, I. D. Campbell, C. Chothia, F. Díaz de Espada, I. Faye, L. García Alonso, P. R. Kolatkar, B. Malissen, C. Milstein, R. Paolini, P. Parham, R. J. Poljak, J. V. Ravetch, J. Salzer, N. E. Simister, J. Trinick, J. Vives, B. Westermark and W. Zimmermann.

9 **Workshop on Control of Gene Expression in Yeast.**
Organized by C. Gancedo and J. M. Gancedo. Lectures by T. G. Cooper, T. F. Donahue, K.-D. Entian, J. M. Gancedo, C. P. Hollenberg, S. Holmberg, W. Hörz, M. Johnston, J. Mellor, F. Messenguy, F. Moreno, B. Piña, A. Sentenac, K. Struhl, G. Thireos and R. S. Zitomer.

10 **Workshop on Engineering Plants Against Pests and Pathogens.**
Organized by G. Bruening, F. García-Olmedo and F. Ponz. Lectures by R. N. Beachy, J. F. Bol, T. Boller, G. Bruening, P. Carbonero, J. Dangl, R. de Feyter, F. García-Olmedo, L. Herrera-Estrella, V. A. Hilder, J. M. Jaynes, F. Meins, F. Ponz, J. Ryals, J. Schell, J. van Rie, P. Zabel and M. Zaitlin.

11 **Lecture Course on Conservation and Use of Genetic Resources.**
Organized by N. Jouve and M. Pérez de la Vega. Lectures by R. P. Adams, R. W. Allard, T. Benítez, J. I. Cubero, J. T. Esquinas-Alcázar, G. Fedak, B. V. Ford-Lloyd, C. Gómez-Campo, V. H. Heywood, T. Hodgkin, L. Navarro, F. Orozco, M. Pérez de la Vega, C. O. Qualset, J. W. Snape and D. Zohary.

12 **Workshop on Reverse Genetics of Negative Stranded RNA Viruses.**
Organized by G. W. Wertz and J. A. Melero. Lectures by G. M. Air, L. A. Ball, G. G. Brownlee, R. Cattaneo, P. Collins, R. W. Compans, R. M. Elliott, H.-D. Klenk, D. Kolakofsky, J. A. Melero, J. Ortín, P. Palese, R. F. Pettersson, A. Portela, C. R. Pringle, J. K. Rose and G. W. Wertz.

13 **Workshop on Approaches to Plant Hormone Action.**
Organized by J. Carbonell and R. L. Jones. Lectures by J. P. Beltrán, A. B. Bleecker, J. Carbonell, R. Fischer, D. Grierson, T. Guilfoyle, A. Jones, R. L. Jones, M. Koornneef, J. Mundy, M. Pagès, R. S. Quatrano, J. I. Schroeder, A. Spena, D. Van Der Straeten and M. A. Venis.

The Centre for International Meetings on Biology has been created within the *Instituto Juan March de Estudios e Investigaciones*, a private foundation which complements the work of the *Fundación Juan March* (established in 1955) as an entity specialized in scientific activities in general.

The Centre's initiatives stem from the Plan for International Meetings on Biology, supported by the *Fundación Juan March*. A total of 30 meetings and 3 Juan March Lecture Cycles, all dealing with a wide range of subjects of biological interest, were organized between 1989 and 1991 within the scope of this Plan.

The Centre endeavours to actively and sistematically promote cooperation among Spanish and foreign scientists working in the field of Biology, through the organization of Lecture and Experimental Courses, Workshops, Seminars, Symposia and the Juan March Lectures on Biology.

Instituto Juan March de Estudios e Investigaciones
Castelló, 77 • Teléfono 34 - 1 - 435 42 40 • Fax 576 34 20
28006 Madrid (España)

The lectures summarized in this publication were presented by their authors at a workshop held on the 19th through the 21th of April, 1993, at the Instituto Juan March.

All published articles are exact reproductions of author's text.

There is a limited edition of 450 copies of this volume, available free of charge.