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Molecular biology of the amyloid of Alzheimer's disease. An overview

NIBALDO C. INESTROSA[1] and CLAUDIO SOTO

Molecular Neurobiology Unit, Department of Cellular and Molecular Biology, Faculty of Biological Sciences, and [1] Joint Program with the Faculty of Medicine, P. Catholic University of Chile, Santiago, Chile

Alzheimer's disease is a progressive neurodegenerative disorder that affects a significant percentage of elderly individuals. Degenerative nerve cells express atypical proteins, and amyloid is deposited. The hallmark event of Alzheimer's disease is the deposition of amyloid as insoluble fibrous masses in extracellular neuritic plaques and around the walls of cerebral blood vessels. This review will focus on the advances on the knowledge of Alzheimer's amyloid, because it is becoming increasingly clear that the deposition of amyloid on neuritic plaques in the brain represents the earliest and most characteristic pathological feature of Alzheimer's disease. The main component of amyloid is a 4.2-4.5 KDa hydrophobic peptide, named amyloid beta-peptide, that is codified in chromosome 21 as part of a much larger precursor protein. The study of the mechanism by which the amyloid beta-peptide arises from the amyloid precursor protein is very important in order to understand the biological basis of amyloid deposition and its role in Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in late life. It is a progresive degenerative disorder of insidious onset, characterized by memory loss, confusion and a variety of cognitive disabilities. Approximately 10% of the population over 65 years old is affected by this form of progressive dementia (Katzman, 1986; Inestrosa, 1992).

The major neuropathological changes in the brains of AD patients were first observed by Alois Alzheimer (1907). Since that time, these abnormalities have been well documented and are clearly visible at the light microscopic level upon autopsy examination. These alterations include neuronal cell death, particularly in regions related to memory and cognition -such as the basal forebrain, hippocampus, limbic and association cortices-, accompanied by the presence of abnormal intra- and extraneuronal proteinaceous filaments in and around surviving neurons (Terry and Katzman, 1983). Intracellularly, bundles of helical and straight filaments, composed largely of phosphorylated ubiquitin-conjugated tau protein and referred to as neurofibrillary tangles, accumulate in large numbers in dying neurons (Kosik, 1991). Extracellularly, amorphous insoluble aggregates of proteinaceous debris, termed "amyloid", appear in the form of senile plaques or neuritic plaques and cerebrovascular amyloid deposits. Abnormal dystrophic neurites are often observed around spherical plaque cores in the cerebral cortex. The frequency and distribution of neurofibrillary tangles and mainly neuritic plaque appear to correlate well with the extent of cognitive impairment, neuronal cell loss, and neurotransmitter depletion, which are also characteristic of AD (Blessed *et al.*, 1968; Glenner, 1980; Terry and Katzman, 1983; Inestrosa, 1992).

Correspondence: Prof. Nibaldo C. Inestrosa, Molecular Neurobiology Unit, Pontifical Catholic University of Chile, P.O. Box 114-D, Santiago 1, Chile. FAX (56-2) 222 5515.

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein.

AMYLOID DEPOSITS

The cognitive dysfunction and memory deficit observed in AD could be directly attributed to neuronal cell death and synapse destruction (Dekosky and Scheff, 1990). In order to determine the primary cause of neuronal cell death, many investigators have focused their attention upon the characteristic lesions of AD. Regions which exhibit major cell loss in AD brains generally include neurofibrillary tangles-containing neurons, cerebrovascular amyloid-rich blood vessels, and neuritic plaques surrounded by spherical aggregates of degenerating neurites (Terry and Katzman, 1983). To date, considerable effort has been directed to the study of the nature of this intra- and extracellular filaments, with the ultimate goal of determining their origin and deciphering their role in the neuropathogenic processes culminating in neuronal cell loss.

The term amyloid was originally coined by Virchow in the nineteenth century, to describe argyrophilic proteinaceous clumps of debris invading the extracellular spaces of tissue. This definition was later expanded to include the specific tinctorial properties of amyloid, including the ability to emit a green birefringent glow after staining with Congo red, and the capacity to bind the fluorochrome, thioflavin S. Both staining properties correlate with the hypothesis of Glenner, that the protein subunits comprising various types of tissue amyloid filaments share a substancial amount of beta-pleated sheet conformation (Glenner, 1980). However, it appears that the shared physical properties of the amyloid proteins may be due to similar intermolecular packing motifs, rather than shared secondary structure (Lansbury, 1992). The amyloid in the neuritic plaque occurs in either tightly compact or loosely amorphous spherical clusters, which are often surrounded by dystrophic nerve terminals containing more than the normal number of lysosomes and mitochondria (Kidd, 1964; Terry *et al.*, 1964; Gonatas *et al.*, 1967). Neuritic plaque are frequently fulfilled with microglia and astroglia, presumably in response to the abnormal proteinaceous debris (Kidd, 1964; Terry *et al.*, 1964; Gonatas *et al.*, 1967).

Immunohistochemical studies have demonstrated a variety of elements residing in or around the plaque core including sulfated glycosaminoglycans found in many types of amyloid (Linker and Carney, 1987), the protease inhibitor alpha-1-antichymotrysin (Abraham *et al.*, 1988), and heparan sulfate proteoglycans (Snow *et al.*, 1988). The presence of proteoglycans was also shown in the non-filamentous preamyloid deposits called "primitive or diffuse plaques", indicating that proteoglycan deposition is probably an early event in the plaque development (Snow *et al.*, 1988).

The main ultrastructural component in the plaque core and cerebrovascular amyloid is a 6-10 nm straight filament, composed of identical subunits in a possible antiparallel array, beta-pleated sheet conformation and exhibiting the staining affinities of amyloid (Glenner and Wong, 1984a). In 1984, Glenner and Wong solubilized the amyloid fibrils from enriched fractions of cerebrovascular amyloid from Down syndrome and AD brains, using guanidine hydrochloride. They purified a 4.2-4.5 KDa hydrophobic peptide which they named the amyloid beta-peptide (Glenner and Wong, 1984a); subsequently, they were able to obtain the aminoacid sequence of the 24 amino terminal residues (Glenner and Wong, 1984b). With the advent of successful protocols for solubilizing plaque core amyloid (formic acid or guanidine thiocyanate), a peptide similar in size and N-terminal sequence to the amyloid beta-peptide in cerebrovascular amyloid was isolated from plaques cores (Masters *et al.*, 1985a).

AMYLOID PRECURSOR PROTEIN

By cDNA cloning, using synthetic oligonucleotide probes based on the published aminoacid sequence of the amyloid beta-peptide, it was possible to isolate complementary DNAs (cDNA) encoding the amyloid beta-peptide as part of a much larger amyloid precursor protein (APP) from human fetal brains cDNAs libraries (Tanzi *et al.*, 1987; Goldgaber *et al.*, 1987; Kang *et al.*, 1987). The full-length cDNA indicates that the amyloid beta-peptide domain is encoded at the carboxyl-terminal end of the APP (Fig. 1) as part of the sole transmembrane domain, in a protein resembling an integral membrane-associated glycoprotein (Kang *et al.*, 1987). The

APP starts with a leader sequence (signal peptide), followed by a cysteine-rich region, an acidic-rich domain, a protease inhibitor motif, a putative N-glycosilated region, a transmembrane domain, and finally, a small cytoplasmatic region. The amyloid beta-sequence begins close to the membrane on the extracellular side and ends part-way through the transmembrane region. Two-thirds of the amyloid beta-peptide reside extracellularly while the other one-third is within the membrane (Fig. 1).

Physical mapping techniques, employing human-rodent somatic cell hybrid lines, have localized the APP gene in the chromosome 21 at the region of 21q11.2 - 21q21 (Tanzi *et al.*, 1987). Subsequent mapping, utilizing *in situ* hybridization and a panel of chromosome 21-specific somatic cell hybrids, revealed that APP resides at the border of band 21q21 and

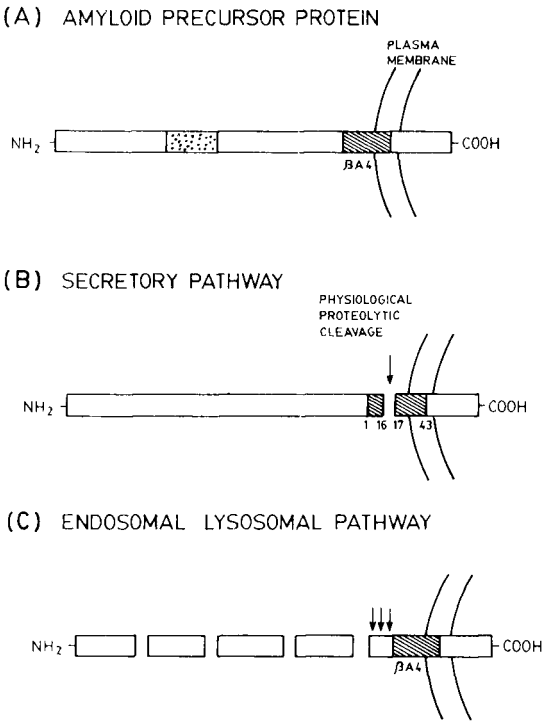

Fig. 1: Squematic representation of APP processing. (A) Domain structure of APP, showing location of amyloid beta peptide (hatched box) and antiprotease Kunitz domain (black box). (B) Secretory pathway for more important physiological processing of APP, which result in release of soluble APP to extracellular space by a membrane bound protease. (C) Endosomal-lysosomal pathway for alternative, less frequent, processing of APP, which result in generation of carboxyl-terminal potentially amiloidogenic fragments. Arrows, putative cleavage sites in endosomal-lysosomal system.

21q22.1 (Patterson *et al.*, 1988). This places APP very close to, if not within, the obligate Down syndrome region, suggesting that plaque formation in people developing Down syndrome at relatively early ages, could be the direct consequence of overproduction of APP, due to an extra dose of the gene. The increased level of APP mRNA in Down syndrome brains (Tanzi *et al.*, 1987) is consistent with this hypothesis, but does not exclude the possibility of intervention of other chromosome 21 loci in the expression or post-translational processing of APP. The APP gene has at least 18 exons which give rise to five differents APP transcripts through alternative splicing. The 3 major transcripts range between 3.4 to 3.6 Kb and code for APPs 695, 751 and 770 amino acids (APP695, APP751, APP770). The two longer forms include a sequence that belongs to the Kunitz family of protease inhibitors (Fig. 1) (Ponte *et al.*, 1988; Tanzi *et al.*, 1988; Kitaguchi *et al.*, 1988). Such antiprotease sequence is almost identical to the so-called protease nexin II (Oltersdorf *et al.*, 1989; van Nostrand *et al.*, 1989). The existence of a functional protease inhibitor domain in APP carries profound implications for the process of amyloidogenesis in AD. The role of the Kunitz-like protease inhibitor domain might be to prevent amyloid formation, by inhibiting specific proteases capable of releasing the amyloid beta-peptide from the precursor. Alternatively, it might accelerate the generation of the amyloid beta-peptide by impeding specific protease from clearing the soluble APP. Recent studies in our laboratory indicates that the APP751, but not the APP695, induces axonal sprouting both in myelinated as well as in nonmedullated axons (Alvarez *et al.*, 1992). In addition, the APP transcript containing the Kunitz protease inhibitor domain modifies the adhesiveness of axons and glial cells and induces the accumulation of an extracellular material (Alvarez, Moreno, Inestrosa and Esch, unpublished observations). Because sprouts of axons and dendrites occur in the periphery of the neuritic plaques it is possible that an extracellular protease-antiprotease system may be altered in AD (Alvarez *et al.*, 1992).

The APP gene expression is ubiquitous, being the higher in brain, kidney, lung, muscle and spleen. In peripheral tissues, the ex-

pression of the two longer transcript is higher than in neurons, whereas the APP695 mRNA is higher in neurons (Cohen *et al.*, 1987). Data supporting increased levels of APP transcripts in AD brain have also been reported (Cohen *et al.*, 1987; Palmert *et al.*, 1988).

Studies of expression of the alternate forms of APP in AD brains have been somewhat contradictory. In one study, *in situ* hybridization revealed a 2-fold increase in levels of APP695 (Cohen *et al.*, 1987); in other, Northern blot analysis portrayed a different picture. While APP695 transcripts appear to be selectively reduced in cerebral cortex, APP751 mRNA levels remained unchanged (Johnson *et al.*, 1988). Quantitative analysis revealed a higher APP770 mRNA level in AD, while the APP695 and APP751 mRNAs levels remains unchanged (Tanaka *et al.*, 1988). On the other hand, studies by the group of Beyreuther and co-workers indicate that alternative splicing of the APP gene in AD is not significantly different from age-matched controls (Konig *et al.*, 1991).

The evidence that amyloid may play an important role in early pathogenesis of AD comes primarily from studies of individuals with Down syndrome. Almost all patients with Down syndrome (trisomy 21) will develop AD neuropathology at an early age. In fact, virtually 100% of the brains examined from middle-aged patients with Down syndrome contain large amounts of neuritic plaques, cerebrovascular amyloid, and neurofibrillary tangles, identical in structure, composition and distribution to those observed in AD patients. In a survey of brains from Down syndrome patients between the ages of 25 and 60 years, diffuse non-filamentous depositions of pre-amyloid were observed in younger subjects in the absence of any neuritic, glial abnormalities and neurofibrillary tangles (Giaccone *et al.*, 1989). These findings have provided the strongest evidence sugesting the idea that amyloid deposition precedes the formation of neuritic plaques in AD. Virtually all patients with Down syndrome develop classical senile plaques, indistinguishable from those with AD if they survive beyond the age of 40.

The aetiology of AD is complex: although a few cases are clearly genetic (autosomal dominant), most are sporadic, and of unknown and probably diverse aetiologies (Katzman and Saitoh, 1991). Segregation analysis of a family in which the pathogenic lesion was known to be on chromosome 21 showed that the APP gene was coinherited with the disease. Sequencing of the APP gene revealed a point mutation (codon 717) in the APP gene (Goate *et al.*, 1991), that results in the substitution of valine for isoleucine in the transmembrane domain of APP, two aminoacids downstream from the carboxy-terminal of the beta peptide sequence. Two additional pathogenic mutations that change valine 717 to glycine (Chartier-Harlin *et al.*, 1991) and phenylalanine (Murrell *et al.*, 1991) have since been characterized. The presence of specific mutations in the APP gene in individuals with familial AD of early onset, provides further evidence that amyloid could play an important role in the pathogenesis of AD. If this scenario proves to be correct, the pathogenetic process leading to AD might be interrupted by drugs that could reduce the production of the APP, or alter the breakdown of APP, thus impeding its accumulation in brain tissue.

PROCESSING OF AMYLOID PRECURSOR PROTEIN

Biochemical studies in cultured cells reveal that APP matures rapidly through a constitutive secretory pathway (Fig. 1). In addition, C-terminally truncated APP derivatives are secreted into the conditioned medium (Weidemann *et al.*, 1989). Molecular biological approaches have been used to demonstrate that APP was cleaved within the amyloid beta-peptide (Sisodia *et al.*, 1990). Analysis of the membrane fragments retained and secreted from cultured mammalian cells showed that APP is cleaved between residues 16 (lysine) and 17 (leucine) of the amyloid beta-peptide (Fig. 1) (Esch *et al.*, 1990). Recent studies indicate that the APP is cleaved on the plasma membrane by a membrane-bound endoprotease (APP secretase) and that the specificity of this peptide bond hydrolysis is largely independent of the primary sequence of the precursor. The principal determinants for the proteolysis appear to be an alpha-helical conformation and the distance (12-13 aminoacid residues) of the hydrolyzed bond from the plasma membrane (Sisodia 1992).

Another amyloid beta-clipping enzyme has been identified, purified and characterized from several sources, including human brain (Tagawa *et al.*, 1991). The abnormal processing of APP to generate amyloid beta-peptide may be explained by an alteration of the APP secretase, or by an AD-specific membrane disturbance, that makes the substrate -APP- accesible to proteinases (Ishiura, 1991). On the other hand, Ishiura *et al.* (1989, 1990) using synthetic peptides have identified a macropain-like multicatalytic proteinase (also known as ingensin) as a candidate for the abnormal APP-processing enzyme. However, no evidence of an increased expression of such proteinase or a decreased inhibitor content has been provided.

Cells also have alternative ways of breaking down APP, and these routes, unlike the secretase reaction, yield fragments (8-12 KDa) that contain intact amyloid beta-peptide and thus have the potential of forming Alzheimer's plaques (Fig. 1) (Estus *et al.*, 1992).

The potentially amyloidogenic derivatives are produced in normal human cells, including brain cells. Moreover, the fragments are formed in lysosomes; in fact, addition of compounds that inhibit lysosomal enzymes (leupeptin and ammonium chloride) decrease the fragments formation (Golde *et al.*, 1992). Previous studies by Cole *et al.* (1989) had shown that inhibitors of lysosomal function increased the surface level of intact APP. Therefore, the APP is normally processed both by the constitutive secretory and by endosomal-lysosomal pathways (Fig. 1).

The hypothesis that APP is processed within lysosomes was previously suggested by electron microscopy studies, after immunostaining with antibodies specific to APP (Benowitz *et al.*, 1989), which showed the existence of APP deposits in secondary lysosomes of CA1 pyramidal cells of the hippocampus. The immunocytochemical detection of ubiquitin (Hardy and Allsop, 1991) and lysosomal proteases (Cataldo and Nixon, 1990) in neuritic plaque also favors the idea that APP processing occurs within the lysosomes.

Very recently, Younkin and coworkers (Shoji *et al.*, 1992) have presented evidence that the amyloid beta-peptide, which is deposited as amyloid in the brains of patients with AD, is normally produced and released as a soluble entity. Moreover, the soluble fragments were detected in cerebrospinal fluid from normal and AD individuals. Thus, it is likely that amyloid deposition in AD involves pathways that normally produce and release amyloid beta-peptide to the extracellular space. The amount of amyloid deposited probably will depend on the rate of amyloid beta-peptide production, its rate of removal and the rate at which soluble beta-peptide forms insoluble amyloid fibrils.

One of the most promising approaches to elucidating the contribution of amyloid to the pathogenesis of AD is the generation of transgenic mice that carry an overabundance of the APP gene. Quon *et al.* (1991) demonstrated accumulation and deposition of amyloid in the mouse brain. Amyloid beta-peptide immunoreactive material was significantly increased in neurons of the brain neuropil as well as in neuronal processes of the hippocampus; full length APP was also present in neuritic process. Immunoreactive deposits were evident in cortex and hippocampus, as both compact and diffuse amorphous deposits (Quon *et al.*, 1991). This work suggests that regulation of the APP expression may be one mechanism to generate amyloid deposits.

Recent studies of Yoshikawa *et al.* (1992) suggest that stable transfectants overexpressing full-length APP could provide an important system to investigate the APP-associated neuropatological features of AD. In fact, to test whether overexpression of APP generates abnormally processed derivatives that affect the viability of neurons, Yoshikawa and co-workers stably transfected full-length human APP complementary DNA into murine embryonal carcinoma P19 cells. These cells differentiate into post-mitotic neurons and astrocytes after exposure to retinoic acid. After differentiation, all neurons showed severe degenerative changes and disappeared within a few days. The degenerating neurons contained large amounts of APP derivatives that were truncated at the amino-terminus and encompassed the entire amyloid-beta peptide. These results suggest that post-mitotic neurons are vulnerable to overexpressed APP, which undergoes aberrant processing to generate potentially amyloidogenic fragments.

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ORIGIN OF THE AMYLOID OF THE SENILE PLAQUES IN ALZHEIMER'S DISEASE

Proponents of the neuronal origin of amyloid plaques argue what amyloid first accumulates intracellularly within neuronal cell bodies and degenerating nerve terminals, in order to form the core of the paired helical filaments of neurofibrillary tangles (Masters *et al.*, 1985b). At a later time, the amyloid would be extruded from the dying neuron into the extracellular matrix to form the amyloid beta-peptide plaque cores. More recently, it has been proposed that the amyloid beta-peptide in neuritic plaques is probably derived from neuronally synthesized APP and deposited at locations remote from sites of synthesis. In fact, the APP is transported within axons by means of the fast anterograde axonal transport (Koo *et al.*, 1990). The fast transport of membranous organelles is one mechanism by which the APP can eventually reach neurites that surround extracellular deposits of amyloid beta-peptide. It is therefore possible that the APP synthesized in neurons would be delivered to dystrophic nerve endings, where some local processing of the precursor could result in the release and deposition of the amyloid beta-peptide into the extracellular space.

Evidence supporting that APP could be synthesized by microglial cells has been accumulated in the last few years. In AD brain, neuritic plaques are consistently found to contain and to be surrounded by microglia (Styren *et al.*, 1990; Perlmutter *et al.*, 1990). Bauer and co-workers (1991) have reported strong constitutive expression of APP in human mononuclear phagocytes after terminal *in vitro* maturation from monocytes to macrophages. In addition, immunocytochemical detection of APP was shown in primary cultures of human microglia. To evaluate the microglial origin of the amyloid, we (Soto and Inestrosa, 1992) have been studying the APP expression in the human monocyte-like cell line U937. In the presence of phorbol esters these cells differentiate to macrophages with the concomitant expression of APP. Because microglial cells derive from blood macrophages, the above results support the idea that blood-derived cells may play a role in the deposition of the amyloid-beta peptide of neuritic plaque.

Terry *et al.* (1987) have reported a subset of clinically typical AD patients with significant amounts of well developed neuritic plaques cores but virtually no neurofibrillary tangles in their cerebral cortices. Non-filamentous pre-amyloid deposits, detected by immunostaining with anti-amyloid antibodies, are neither argyrophilic, congophilic, nor thioflavin S-positive and are found in the absence of neurofibrillary tangles and plaques in the cortex of AD patients (Yamaguchi *et al.*, 1988; Tagliavini *et al.*, 1988).

Wisniewski *et al.* (1988) have reported that young patients with Down syndrome exhibit abundant amyloid plaques in the cerebral cortex, but no neurofibrillary tangles. All these observations strongly argue against the hypothesis that intracellular neurofibrillary tangles give rise to neuritic plaques cores.

AMYLOID INDUCES BOTH NEUROTOXIC AND NEUROTROPHIC ACTIVITY IN THE BRAIN

Neuritic plaques-associated dystrophic neurites of various neurotransmitter types are accumulated around plaque cores, as if they were attracted to a focal point of trophic activity (Geddes *et al.*, 1985; Walker *et al.*, 1988). Evidence of abnormal neuritic sprouting, as well as increased levels of trophic activity, have been reported to occur in AD brains (Uchida *et al.*, 1988; Whitson *et al.*, 1989; Yankner *et al.*, 1990). In fact, a synthetic peptide including aminoacids 1-28 of the amyloid beta-peptide is able to sustain the survival of primary rat hippocampal cultures and also appears to function as a neurite promoting factor (Whitson *et al.*, 1989). These results suggest that focal depositions of amyloid in the brain may induce a trophic response in the neurons. This might explain both the appearance of abnormal dendritic arborization around the cell body, and the presence of numerous neurites extending into the plaque cores. Neurites at the site of the plaque may mark the initial contact of the neuron with a focal deposit of amyloid. Yankner *et al.* (1990) have shown that a synthetic peptide encoding the first 40 residues of the amyloid beta-peptide has both trophic and toxic effects on cultured fetal rat hippocampal neurons. In fact, the amyloid beta-peptide was neurotrophic to

undifferentiated neurons at low concentrations, and neurotoxic to mature neurons at high concentrations, causing dendritic and axonal retraction followed by neuronal death. A portion of the beta-peptide (aminoacids 25 to 35) mediated both the trophic and toxic effects, and was homologous to the tachykinin neuropeptide family. The effects of the amyloid beta peptide were mimicked by tachykinin antagonists and completely reversed by specific tachykinin agonists (substance P) (Yankner *et al.*, 1990). The amyloid beta-peptide also sensitizes mouse cultured cortical neurons to the toxic effect of agonists of the glutamate receptor (Koh *et al.*, 1990). Further evidence suggests that amyloid precursor fragments may be neurotoxic. In fact, the medium conditioned by PC12 cells transfected with a fragment of APP gene containing the putative intracellular domain (C terminus), is toxic to cultured central neurons. These findings suggest that aberrant processing of APP may produce toxic peptides in AD (Yankner *et al.*, 1989). Calcium ions participate both in glutamate neurotoxicity (Choi, 1987) and in the neurite outgrowth process (Mattson and Kater, 1987). Therefore, it is possible that the amyloid beta-peptide may alter calcium metabolism in such a way as to render mature cortical neurons more vulnerable to damage and degeneration. Indeed, recent studies in human cerebral cortical cell cultures strongly suggest that amyloid beta-peptide destabilizes neuronal calcium homeostasis and thereby renders neurons more vulnerable to environmental insults (Mattson *et al.*, 1992). A 4 day exposure to amyloid beta-peptide alone had no effect on neuronal survival, but enhanced both kainate and NMDA neurotoxicity, indicating that the effect was not specific for a particular subtype of glutamate receptor. The effect required prolonged (several days) exposures. The neurotoxicity caused by excitatory aminoacids and potentiated by amyloid beta-peptide was dependent upon calcium influx, since it did not occur in calcium-deficient culture medium. Direct measurements of intracellular calcium levels demonstrated that amyloid beta-peptide elevated rest levels of calcium and enhanced calcium responses to excitatory aminoacids and calcium ionophore. Finally, the amyloid beta-peptide made neurons more vulnerable to neurofibrillary tangles-like antigenic changes induced by excitatory aminoacids (Mattson *et al.*, 1992).

CONCLUDING REMARKS

Over the past eight years, a significant progress in the understanding of many features of AD has been reached, including the identification of the molecular components of the amyloid. The recent discovery of a pathogenic mutation of the APP gene at codon 717 on chromosome 21, suggests that APP mismetabolism and amyloid beta-peptide deposition are the primary events in the disease process. The common ocurrence of AD in Down syndrome patients is consistent with this hypothesis. The biological basis of amyloidogenesis will be clarified by studies focused on the cellular and molecular biology of APP. In particular, it will be important to use cultured cells to identify the specific pathway and proteases that produce and release the amyloid beta-peptide, and the factors that foster amyloid beta-peptide production. In addition, it will be important to identify the factors that cause soluble extracellular amyloid beta-peptide to form amyloid fibrils. All these informations may well provide crucial advances concerning the molecular basis of AD and the treatment of this highly prevalent and disabling disorder.

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