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The Molecular Pathology of Amyloid Deposition in Alzheimer's Disease

Ralph N. Martins,*[*,1] Phillip J. Robinson,[2] John O. Chleboun,[1] Konrad Beyreuther,[3] and Colin L. Masters[4]

[1]University Department of Surgery, Repatriation General Hospital, Hollywood, Perth Western Australia 6009; [2]John Hunter Hospital, Endocrine Unit, Locked Bag 1, Newcastle Mail Centre, New South Wales 2310; [3]Centre for Molecular Biology, The University of Heidelberg, 6900 Heidelberg, Federal Republic of Germany; and [4]Department of Pathology, The University of Melbourne and the Mental Health Research Institute of Victoria, Parkville, Victoria 3052

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Introduction

Alzheimer's disease (AD) is the most common form of cerebral degeneration leading to dementia. The disease can only be conclusively diagnosed at death following postmortem examination of the brain for the presence of amyloid deposited in the amyloid plaque core (APC), the neurofibrillary tangles (NFT), and in and around cerebral blood vessels as amyloid congophilic angiopathy (ACA). AD sufferers can be placed in three major groups: Group 1 individuals suffer from Downs syndrome (DS), where the pathological changes of AD are found in all cases over the age of 40 (Wisniewski and Rabe, 1986); Group 2 individuals suffer from early onset familial AD, where a rapidly progressive dementia occurs leading to death in the fifth decade with massive neuropathological changes; the third group is less well-defined and contains the more common later onset cases, both sporadic and familial, the course of which illness is much more variable and exhibits a wide range of neuropathological severity. The development of the clinical features of AD is

*Author to whom all correspondence and reprint requests should be addressed.

linked to the amount of deposition of amyloid in the limbic areas and cerebral cortex. The major component of the amyloid deposits present in the APC, NFT, and ACA is a unique peptide termed βA4, which has a molecular mass of 4 kDa (Allsop et al., 1983; Glenner and Wong, 1984a; Masters et al., 1985a; Selkoe et al., 1986; Prelli et al., 1988a; Roher et al., 1988; Guiroy et al., 1987; Shapira et al., 1988). Subsequent complete sequencing showed that the βA4 protein consists of 42 or 43 residues (Fig. 1), the C terminal, 12 of which are hydrophobic.

These hydrophobic residues of the βA4 protein confer on it the ability to self-aggregate and polymerize into amyloid fibrils (Masters et al., 1985a,b; Beyreuther et al., 1986).

Molecular Forms of the Amyloid Precursor Protein

The isolation of full-length cDNA clones that code for βA4 revealed that this peptide was derived from a much larger precursor termed the amyloid precursor protein (APP), and its gene was localized to the long arm of chromosome 21 (Donnelly et al., 1988; Goldgaber et al., 1987; Kitaguchi et al., 1988; Lovett et al., 1987; Patterson et al., 1988; Ponte et al., 1988; Robakis et al., 1987; Shivers et al., 1988; Tanzi et al., 1987a; Yamada et al., 1987; Zabel et al., 1989; Zain et al., 1988). There are at least five different forms of APP that are obtained by differential splicing from the same gene (Kang et al., 1987; Kitaguchi et al., 1988; Ponte et al., 1988). These forms are APP-563, APP-695, APP-714, APP-751, and APP-770 (Fig. 2). APP-563, APP-751, and APP-770 have in common a protein domain that has high homology with the kunitz protease inhibitors (KPI) (Kunitz, 1947; Laskowski and Kato, 1980). The Kunitz family of serine protease inhibitors are potent inhibitors of trypsin and contain six cysteine residues that are conserved. In addition, a basic amino acid is present in the active center of these inhibitors. The best-studied member of this family is pancreatic trypsin inhibitor, commonly called aprotinin. The KPI domain of APP is 50% identical to aprotinin and also to the second inhibitory domain of the human plasma protein, inter-α-trypsin inhibitor. The molecular forms of APP containing the KPI domain have also been shown to be potent inhibitors of trypsin-like activity (Kitaguchi et al., 1988; Castro et al., 1990; Sinha et al., 1990) and are the main forms in all tissues except the brain. All the APP forms with the exception of APP-563 have the structural domains of integral transmembrane cell surface receptors (Kang et al., 1987). These molecules cross the bilipid layer once and have a short cytoplasmic tail (Kang et al., 1987; Dyrks et al., 1988). The βA4 sequence lies partly in the extracellular domain and also extends into the transmembrane region. The βA4 and cytoplasmic domains of APP show a propensity to aggregate to form a complex with a novel resistance to protease attack (Weidemann et al., 1989).

Some investigators have postulated that the higher ratios of the protease inhibitory containing forms of APP to APP-695 observed in the aging brain play a role in βA4 production by altering the normal processing of APP (Kitaguchi et al., 1988; Johnson et al., 1990). Support for the above postulation has come from the recent work of Quon et al. (1991), who have demonstrated βA4 immunoreactive deposits in brains of transgenic mice expressing increased levels of APP 751.

Genetics of the Familial Alzheimer's and APP Gene

The link between DS and AD was strengthened when it was demonstrated that the APP gene resides on chromosome 21 immediately proximal to the obligate DS region in bands 21q21.105–21q21.2 (Korenberg et al., 1988). The finding of the APP gene on chromosome 21 led to speculation that patients with AD might have a microduplication of a subsequence of chromosome 21 containing the APP gene (Kitaguchi et al., 1988). However, studies from three laboratories have not substantiated this notion (Goedert,

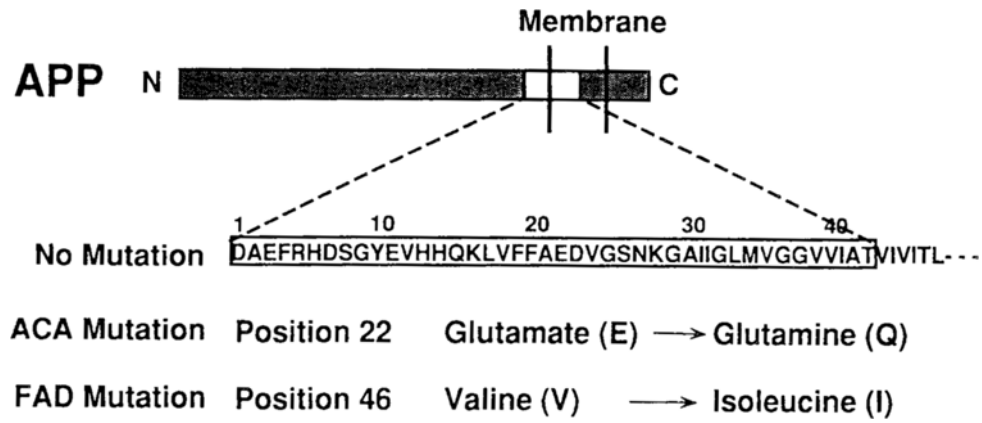

Fig. 1. Amino acid sequence of βA4 and its location in APP. βA4 is composed of 43 amino acids (enclosed sequence), 28 of which are in the extracellular domain and 15 in the transmembrane domain. The Dutch form of familial amyloid congophilic angiopathy (ACA) is a result of a mutation at position 22 of βA4. The mutation detected in some familial Alzheimer's disease pedigrees is at position 46 of the transmembrane domain of APP. The amino acid numbering is based on position 1 corresponding to the first amino acid in the βA4 sequence.

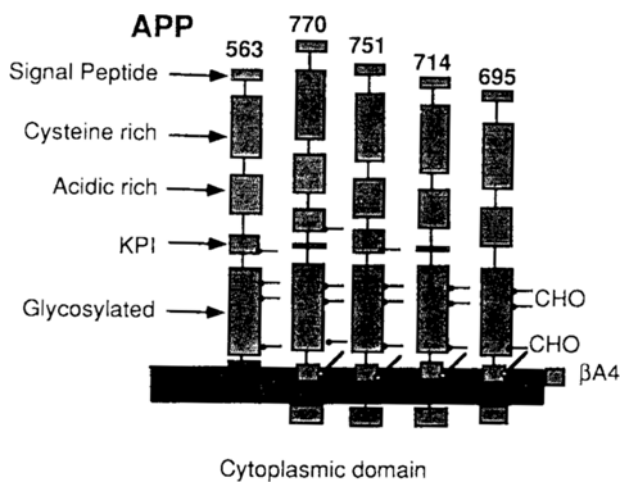

Fig. 2. The structural domains of the five molecular forms of APP. The five isoforms of APP are designated APP563, APP770, APP751, APP714, and APP695 as predicted from the amino acid sequence translated from their respective cDNAs. The number associated with each of the APP molecules corresponds to the number of amino acids characteristic of each isoform. The domains designated OX and KPI have homology with the Ox-2 antigen and the Kunitz family of serine protease inhibitors, respectively. CHO represents glycosylation sites for APP.

1987; Dyrks et al., 1988; Shivers et al., 1988). Furthermore, consistent with the above reports, we have shown that DS tissues, but not AD tissues, have a 50% increase in APP levels above controls (Rumble et al., 1989). Therefore, APP gene duplication and increased APP levels may not be the molecular mechanisms leading to βA4 deposition in AD.

Chromosome 21 was also shown to contain the familial Alzheimer's disease (FAD) gene (St. George-Hyslop et al., 1987), which is inherited in an autosomal dominant manner in early onset pedigrees (<65 yr). Further analyses indicated that the FAD locus and the APP gene are distinct and not linked (Van Broeckhoven et al., 1987; Tanzi et al., 1987b, Tanzi, 1991). Extended linkage studies reveal that most FAD early onset pedigrees map to chromosome 21, but late onset FAD pedigrees generally do not (Tanzi, 1991). The above study indicates that FAD results from genetic defects on other chromosomes in addition to chromsome 21. Although FAD and the APP genes are not linked, the close proximity of these loci on chromosome 21 (<1% of the human genome) suggests that an interaction between them cannot be excluded at this stage.

Recently, Goate et al. (1991) have reported in two FAD families a point mutation in exon 17 of APP, resulting in a valine to isoleucine substitution at residue 717 (APP 770), which is positioned within the transmembrane segment, only two residues beyond the C terminus of βA4 (Fig. 1). This finding has led to the hypothesis that this mutation is pathogenic. The occurrence in other families with AD from Japan and France with residue 717 mutation lends credence to the above hypothesis (Hardy et al., 1991). However, this mutation cannot be the cause of all forms of FAD, since out of 143 pedigrees studied to date, only 7 were positive for the valine to isoleucine substitution at residue 717 (Tanzi, 1991). It is also not clear how the results of Goate et al. (1991) can be accommodated with the multiple crossover events between FAD and APP in families linked to chromosome 21 markers (Tanzi et al., 1987b; Van Broeckhoven et al., 1987). Wright et al. (1991) have suggested that there must be another gene on chromosome 21, nearer to the centromere that predisposes to FAD. Alternatively, reports of recombination between familial AD and the APP gene in chromosome 21-linked families may have been in error resulting from nonpaternity, mistyping, misdiagnosis, or phenocopies (Goate et al., 1991).

In hereditary cerebral hemorrhage with amyloidosis of the Dutch type (HCHWA-D), an autosomal dominant form of cerebral amyloid angiopathy, a glutamate-to-glutamine substitution occurs at position 22 of βA4 (Van Broeckhoven et al., 1990). In this disease, in contrast to FAD, βA4 deposits are predominantly in cerebral blood vessels and only small numbers in the neuropil, with no NFT. If this mutation is the cause of HCHWA-D, as supported by the linkage data (Van Broeckhoven et al., 1990), then taken together with the FAD mutation, it could be suggested that different mutations in the APP molecule may result in βA4 deposition in some anatomical regions of the brain, but not others, because of the site-specific complement of endogenous proteases. The heterogeneity of the neuropathology seen in AD may thus be explained should a number of new APP mutations be identified exhibiting mutation-specific neuropathology.

Biochemistry of APP

Although the function(s) of the APP isoforms have not been clearly established, it appears that APP may have a role in cell growth and neuronal development (Milward et al., 1991; Roch et al., 1991), which does not require the kunitz protease inhibitor (KPI) domain for its activity. We and others have demonstrated that the KPI-containing form of APP is abundant in the α granule of platelets (Bush et al., 1990; Van Nostrand et al., 1990) and released in response to platelet activators. This form of APP has been identified with previously known molecules, such as protease nexin 2 (Van Nostrand et al., 1990), the inhibitor of coagulation factor XIa (Smith et al., 1990), or a heparin binding molecule (Schubert et al., 1989). Since protease nexin 2 binds to the γ subunit of nerve growth factor, the epidermal growth factor binding protein, and transforming growth factor β, this blood form of APP may play a key role in wound healing.

APP is expressed throughout the brain in nonneural tissues and cultured cells as assessed by mRNA and protein analyses (Tanzi et al., 1987a; Goedert, 1987; Bahmanyar et al., 1987; Shivers et al., 1988; Weidemann et al., 1988; Selkoe et al., 1988; Rumble et al., 1989; Martins et al., 1990; Bush et al., 1990; Moir et al., 1991). We have isolated and sequenced the N-terminal regions of the 80-, 100–110, and 120–130-kDa forms of APP from human brain and platelets (Martins et al., 1990; Bush et al., 1990; Moir et al., 1991). The human brain APP forms were similar between control and AD. Postmortem tissue had higher levels of the 80 kDa form and less of the 120–130 kDa form when compared with biopsy tissue. This change was paralleled by increased C-terminal truncation of the membrane-associated forms of APP in postmortem human brain (Martins et al., 1990; Moir et al., 1991). The C-terminal fragments generated by the partial breakdown

of the 120–130-kDa APP form could not be detected by our 22C11 monoclonal antibody, since it is directed against the N-terminus of APP. However, Selkoe et al. (1988) have identified an 11-kDa APP fragment in brain using C-terminal antibodies directed against APP corresponding to the region from amino acid 592 to amino acid 695 (Kang et al., 1987). Since βA4 of APP695 corresponds to residues 596–635, the 11-kDa region may include βA4. Pasternock et al. (1991), using Tris/Tricine 10–16% SDS PAGE gels, which gives better resolution, have identified a complex set of small-mol-wt C-terminal fragments. The authors did not find any differences between APP C-terminal fragments from control and AD brain. The latter observation is not surprising, since Esch et al. (1990) have shown that APP is normally processed by an enzyme (termed secretase) that cleaves it in the middle of its βA4 domain to generate a secreted form of the molecule (Fig. 3). Thus, the normal cleavage of βA4 at residue 16 by secretase (Esch et al., 1990) may result in a number of C-terminal fragments that might mask the detection of the minor full-length βA4 derivatives generated in AD brain. Enhanced resolution, such as 2-D electrophoresis, followed by more sensitive immunoassays may help in the identification of C-terminal fragments containing full-length βA4 in AD brain. However, Pasternock et al. (1991) did identify at least one C-terminal fragment that appears to contain full-length βA4 by immunoassay. Conclusive evidence must await protein sequencing of these C-terminal fragments.

A lot of effort is currently being directed towards identifying the proteases that cleave APP to release βA4. Recently Abraham et al. (1991a,b) have elegantly identified a calcium-activated serine protease from human brain that can cleave APP at three locations, including between methionine and aspartic to generate the N terminus of βA4. These authors have also isolated this protease from perfused fresh-frozen monkey brain, suggesting that it is brain derived. It will be interesting to determine levels of this protease in control and AD brain. Although it is important to study brain proteases to gain insight into βA4 generation, structural changes in the APP molecule (in addition to point mutations) itself may also play a key role. We and others have demonstrated that APP is glycosylated in a stepwise fashion and sulfated (Weidemann et al., 1989, Oltersdorf et al., 1990), and that it can be phosphorylated (Gandy et al., 1988; Martins and Robinson, unpublished data). We have confirmed Gandy et al.,'s (1988) finding that C-terminal APP peptide corresponding to amino acid residues 645–661 (Kang et al., 1987) was phosphorylated by protein kinase C and not by protein kinase A, and have shown with C-terminal truncated human brain APP that this action of protein kinase C was specific for the C terminus of APP. We have also demonstrated that both protein kinase A and endogenous neuronal kinase(s) phosphorylate the 100–110 kDa human brain APP on common and specific sites (Martins and Robinson, unpublished data). Studies to compare the phosphorylation of APP from control and AD brain are currently under way. Gandy et al. (1988) suggested that the state of phosphorylation might regulate its rate of internalization and metabolic disposition, as has been shown for the EGF receptor and IL-2 receptor. Thus, abnormal phosphorylation of APP is a possible candidate for one of the molecular processes in the generation of βA4. Taken together, the evidence indicates that altered posttranslational modification of APP in AD may result in the action of protease(s) at normally noncleaved sites on this protein to release βA4 (Fig. 3).

Conclusion

It is evident that, although an excessive production of APP may be the principal cause of βA4 generation in DS, other processes either singly or in combination must be considered to explain the neuropathological heterogeneity characteristic of AD. These include:

1. Syntheses of APP variants;
2. Altered secretase activity;

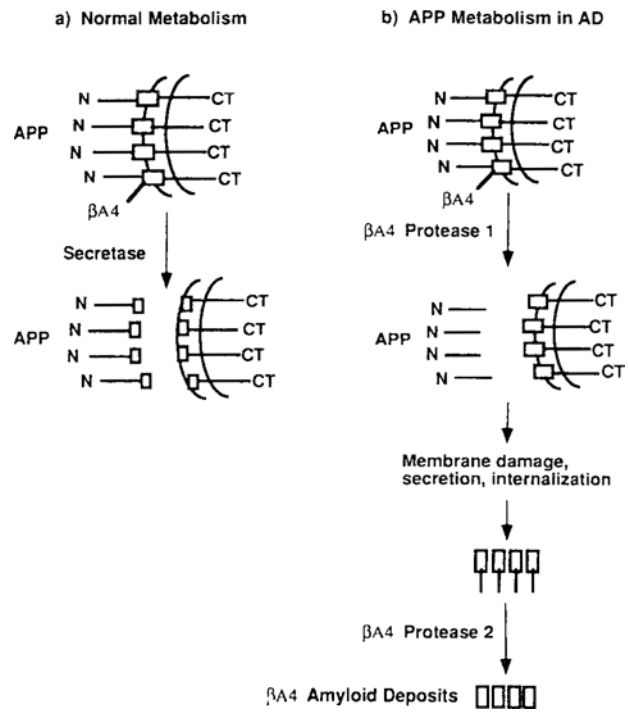

Fig. 3. Proposed schematic illustration of the metabolism of APP in normal and disease states. APP is normally cleaved by an enzyme termed secretase at position 16 of the βA4 sequence. **(a)** In AD, intact βA4 may be released by the action of two proteases. **(b)** βA4 proteases 1 and 2 are postulated here to act at the N and C termini of βA4, respectively.

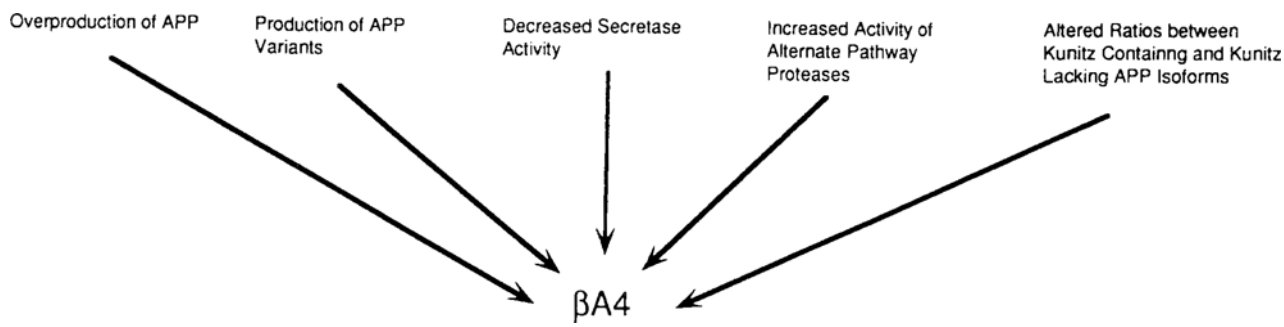

Fig. 4. Proposed molecular mechanisms for βA4 generation. βA4 may be generated by: (1) the excessive production of APP in the brain resulting in the constitative pathway being overloaded; (2) alteration in the structure of APP by either point mutations or posttranslational processing, resulting in resistance to the normal secretase activity; (3) decreased secretase activity in AD; (4) increased activity of proteases that release full-length βA4 in AD; and (5) increased ratios of the KPI-containing form of APP to the APP 695 form, resulting in inactivation of the normal secretase activity.

3. Modified activity of alternate pathway proteases; and
4. Altered ratios between kunitz-containing and kunitz-deficient isoforms (Fig. 4).

Of particular significance is the recent finding of a calcium-activated serine protease that cleaves APP one amino acid upstream from βA4 (Abraham et al., 1991a). This enzyme may cata-

lyze the first step of the alternative pathway when APP is altered posttranslationally or when the secretase activity is decreased in AD.

Molecular biochemical technology has contributed substantially to recent advances in the understanding of this disease. However, further study of the pathological processes operative in Alzheimer's disease is required before effective therapy can be developed.

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