Alzheimer's Disease: The Amyloid Cascade Hypothesis

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Alzheimer's disease causes dementia in many elderly people and in some individuals with Down syndrome who survive to age 50. Alzheimer's is characterized by various pathological markers in the brain—large numbers of amyloid plaques surrounded by neurons containing neurofibrillary tangles (*1*), vascular damage from extensive plaque deposition (*2*), and neuronal cell loss (*1*). Because it is not known if the amyloid plaques or the neurofibrillary tangles are the earliest lesion in the disease process, the role of these markers in the etiology of the disease is controversial.

Our hypothesis is that deposition of amyloid β protein (AβP), the main component of the (*3*) plaques, is the causative agent of Alzheimer's pathology and that the neurofibrillary tangles, cell loss, vascular damage, and dementia follow as a direct result of this deposition. AβP is a peptide product of the larger amyloid precursor protein (APP) (*4*). Because Down syndrome is caused by trisomy of the region of chromosome 21 that contains the APP gene, deposition of AβP is likely to be an early event in the disease (*5*). The AβP molecule is a 39– to 42–amino acid peptide (*4, 6*), part of which forms the hydrophobic transmembrane domain in the COOH-terminal portion of APP (Fig. 1). AβP is one of a diverse group of "amyloid" (starch-like) proteins that forms insoluble extracellular deposits. The APP gene undergoes alternative RNA splicing to produce several protein isoforms; the predominant variant in brain lacks a serine protease inhibitor domain that is present in APP molecules in other tissues (*7*).

We now know something about how APP proteolysis leads to AβP deposition. APP is inserted into the cytoplasmic membrane and then cleaved at residues 15 to 17 within the AβP sequence by the APP "secretase" (*8*) (Fig. 1). This cleavage event therefore produces fragments that do not contain intact AβP and so cannot result in amyloid deposition. These fragments include secreted NH_2-terminal derivatives that can be detected in brain and cerebrospinal fluid (*9*). The APP secretase that cuts within the AβP region has an extraordinarily broad sequence specificity and recognizes the secondary structure of APP, cleaving at a defined distance from the membrane (*10*). Several recent studies suggest that APP can also be processed by the endosomal-lysosomal pathway, after recycling of membrane-bound APP and possibly via an intracellular metabolic route (*11–13*). Carboxyl-terminal fragments containing the entire AβP sequence can be derived from this alternate normal processing of APP (*12, 14*) and may eventually lead to amyloid deposition (*12, 14*) (Fig. 1).

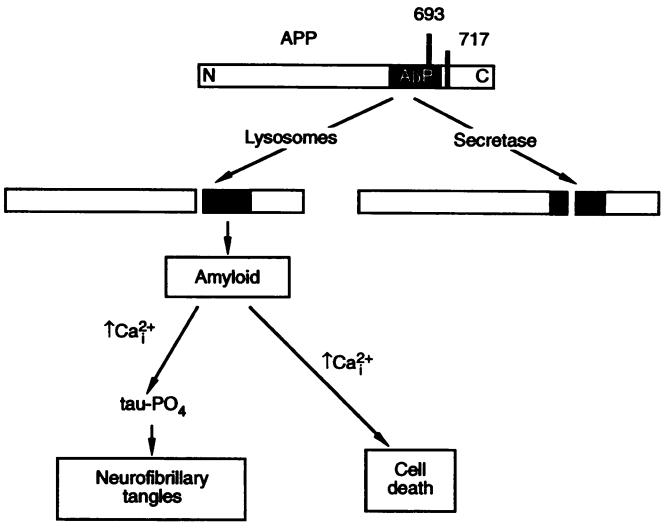

Fig. 1. The amyloid cascade hypothesis. Processing of APP can occur via two pathways: (i) Cleavage within AβP by the secretase, which generates peptide products that do not precipitate to form amyloid and (ii) cleavage in the endosomal-lysosomal compartment, resulting in intact AβP that precipitates to form amyloid and, in turn, causes neurofibrillary tangles and cell death, the hallmarks of Alzheimer's disease.

Mutations in the COOH-terminal portion of APP cause hereditary, early onset Alzheimer's disease (*15, 16*) and hereditary cerebral hemorrhage with amyloidosis (Dutch-type) (*17*). The APP mutation that causes massive AβP deposition in the Dutch amyloidopathy is a glutamic acid to glutamine substitution at codon 693 [with reference to the longest form of APP, APP-770 (*7*)] (Fig. 1), located only six residues away from the cleavage site within the AβP sequence (*17*). It has been speculated that this mutation might cause AβP deposition by inhibiting secretase cleavage of APP, although this now seems less likely because of the apparent lack of sequence specificity of the enzyme (*10*).

Three mutations have been described within the APP gene that cause familial Alzheimer's disease. These mutations all occur at codon 717 of the protein (*15, 16*) and change the native valine, located three residues from the COOH-terminal end of AβP, to isoleucine, phenylalanine, or glycine (Fig. 1). It is unclear how these mutations cause amyloid deposition, but they may inhibit the breakdown of a COOH-terminal fragment of APP that contains AβP (*15*), alter the anchoring of APP in the cell membrane, or stabilize AβP-containing amyloidogenic fragments within lysosomes (*12, 15*).

Our cascade hypothesis states that AβP itself, or APP cleavage products containing AβP, are neurotoxic and lead to neurofibrillary tangle formation and cell death. Thus, two successive events are needed to produce Alzheimer's pathology. First, AβP must be generated as an intact entity, either by accumulation of AβP or as an AβP-containing fragment of APP. Second, this molecule must facilitate or cause neuronal death and neurofibrillary tangle formation. Neve and her colleagues have reported that the AβP-containing COOH-terminal fragment is toxic to cultured neurons (*18*), and Kowall and co-workers (*19*) have suggested that AβP alone exerts toxic effects on neurons, an effect possibly mediated through the serpin receptor (*20*). Other investigators, however, have reported that AβP itself is not neurotoxic, but that it renders neurons more sensitive to excitotoxic damage (*21*). Although it is not clear exactly how AβP causes neuronal loss and tangle formation, the peptide is known to disrupt calcium homeostasis and increase intraneuronal calcium concentrations (Fig. 1). This observation could explain how neurofibrillary tangles form. The tangles are largely composed of paired helical filaments formed from a hyperphosphorylated form of the microtubule associated protein, tau (*6*),

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and tau phosphorylation can be controlled by intracellular calcium (*22*). Thus, AβP may induce neurofibrillary tangle formation as a consequence of its ability to increase the concentration of intracellular calcium, leading to phosphorylation of tau and the formation of paired helical filaments (*6*). The intervening steps by which AβP affects calcium homeostasis still remain to be elucidated. However, the overall mechanism is consistent with what we know about calcium-mediated neuronal death.

The mutations in APP so far described are responsible only for a small proportion of cases of Alzheimer's disease (*23*). Indeed, most cases of Alzheimer's seem to occur in a sporadic fashion, suggesting that there must be other causes of the disease. The cascade hypothesis suggests that other causes of Alzheimer's act by initially triggering AβP deposition. For example, there is an association between head trauma and Alzheimer's (*24*). Dementia pugilistica, exhibited by boxers, may be thought of as a variant of Alzheimer's disease because these individuals exhibit both AβP deposits and neurofibrillary tangles (*25*). Furthermore, amyloid deposition occurs as an acute response to neuronal injury in both man and animals (*26*). This deposition could be caused by an induction of the APP gene through an interleukin-mediated stress response (*27*) because APP increases in response to a number of neuronal stresses (*28*). Although acute effects may only lead to transitory disruption of APP metabolism, it is possible that in some individuals the entire pathological cascade leading to Alzheimer's would be initiated.

The evidence we have described supports the hypothesis that the AβP molecule initiates the pathological cascade of Alzheimer's disease. AβP-containing COOH-terminal derivatives of APP seem the most likely molecular candidates for initiation of the cascade, with the process presumably taking several decades to produce the full-blown pathology of the disease. The ongoing development of transgenic animals that express APP or AβP and exhibit Alzheimer's-like pathology should provide good models for experimental testing of key elements in the cascade. The identification of additional mutations in APP and other genes that cause Alzheimer's pathology will allow refinement of the amyloid cascade hypothesis and point to targets for therapeutic intervention.

REFERENCES

1. R. Katzman and T. Saitoh, *FASEB J.* **5**, 278 (1991); D. M. Mann and M. M. Esiri, *J. Neurol. Sci.* **89**, 169 (1989); D. J. Selkoe, *Neuron* **6**, 487 (1991).
2. G. Blessed, B. E. Tomlinson, M. Roth, *Br. J. Psychiatry* **114**, 797 (1968).
3. G. Glenner and C. W. Wong, *Biochem. Biophys. Res. Commun.* **120**, 885 (1984); *ibid.* **122**, 1131 (1984); C. L. Masters *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4245 (1985); D. J. Selkoe, C. R. Abraham, M. B. Podlinsy, L. K. Duffy, *J. Neurochem.* **146**, 1820 (1986).
4. J. Kang *et al.*, *Nature* **325**, 733 (1987); D. Goldgaber, M. I. Lerman, O. W. McBride, U. Saffiotti, D. C. Gajdusek, *Science* **235**, 877 (1987); N. K. Robakis, N. Ramakrishna, G. Wolfe, H. M. Wisniewski, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 4190 (1987); R. E. Tanzi *et al.*, *Science* **235**, 880 (1987).
5. D. M. Mann *et al.*, *Neuropathol. Appl. Neurobiol.* **15**, 317 (1989); B. Rumble *et al.*, *N. Engl. J. Med.* **320**, 1446 (1989).
6. V. M.-Y. Lee, B. J. Balin, L. Otvos, Jr., J. Q. Trojanowski, *Science* **251**, 675 (1991); K. S. Kosik *et al.*, *Neuron* **1**, 817 (1988); M. Goedert, M. G. Spillantini, R. Jakes, D. Rutherford, R. A. Crowther, *ibid.* **3**, 519 (1989).
7. N. Kitaguchi, Y. Takahashi, Y. Tokushima, S. Shiojiri, H. Ito, *Nature* **331**, 530 (1988); P. Ponte *et al.*, *ibid.*, p. 525; R. E. Tanzi *et al.*, *ibid.*, p. 528; J. Kang and B. Muller-Hill, *Biochem. Biophys. Res. Commun.* **166**, 1192 (1990); K. Yoshioka, T. Miki, T. Katsuya, T. Ogihara, Y. Sakaki, *ibid.* **178**, 1141 (1991); S. A. Johnson, T. McNeill, B. Cordell, C. E. Finch, *Science* **248**, 854 (1990); R. L. Neve, J. Rogers, G. A. Higgins, *Neuron* **5**, 329 (1990).
8. S. S. Sisodia, E. H. Koo, K. Beyreuther, A. Unterbeck, D. L. Price, *Science* **248**, 492 (1990); F. S. Esch *et al.*, *ibid.*, p. 1122; J. P. Anderson *et al.*, *Neurosci. Lett.* **128**, 126 (1991).
9. M. R. Palmert *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6338 (1989); D. Schubert, M. LeCorbiere, T. Saitoh, G. Cole, *ibid.*, p. 2066; A. Weidemann *et al.*, *Cell* **57**, 115 (1989).
10. S. Sisodia, *J. Cell Biol.* **115**, 61a (1991).
11. D. O. Wirak *et al.*, *Science* **253**, 323 (1991); F. A. Sandhu, M. Salim, S. B. Zain, *J. Biol. Chem.* **266**, 21331 (1991); B. D. Greenberg *et al.*, *Soc. Neurosci. Abstr.* **17**, 913 (1991).
12. S. Estus *et al.*, *Science* **255**, 726 (1992); T. E. Golde, S. Estus, L. H. Younkin, D. J. Selkoe, S. G. Younkin, *ibid.*, p. 728.
13. C. Haass, A. Y. Hung, D. J. Selkoe, *J. Neurosci.* **11**, 3783 (1991).
14. C. Nordstedt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 8910 (1991); S. Gandy *et al.*, *J. Cell Biol.* **115**, 122a (1991).
15. A. Goate *et al.*, *Nature* **349**, 704 (1991); J. Hardy *et al.*, *Lancet* **337**, 1342 (1991); M.-C. Chartier-Harlin *et al.*, *Nature* **353**, 844 (1991).
16. S. Naruse *et al.*, *Lancet* **337**, 978 (1991); J. Murrell, M. Farlow, B. Ghetti, M. D. Benson, *Science* **254**, 97 (1991).
17. C. Van Broeckhoven *et al.*, *Science* **248**, 1120 (1990); E. Levy *et al.*, *ibid.*, p. 1124.
18. B. A. Yankner *et al.*, *ibid.* **245**, 417 (1989); R. L. Neve *et al.*, in *Molecular and Cellular Mechanisms of Neuronal Plasticity in Normal Aging and Alzheimer's Disease*, P. D. Coleman, G. A. Higgins, C. H. Phelps, Eds. (Elsevier, Amsterdam, 1990), pp. 257–267.
19. N. W. Kowall, M. F. Beal, J. Busciglio, L. K. Duffy, B. A. Yankner, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7247 (1991).
20. A. Roher, E. G. Gray, M. Paula-Barbosa, *Proc. R. Soc. London, Ser. B* **232**, 367 (1988); H. M. Wisniewski, A. W. Vorbrodt, J. Weigel, J. Morys, A. S. Lossinsky, *Am. J. Med. Gen.* **7**, 287 (1990).
21. J. Koh, L. L. Yang, C. W. Cotman, *Brain Res.* **533**, 315 (1990); M. P. Mattson *et al.*, *J. Neurosci.* **376** (1992); M. P. Mattson, *Neuron* **4**, 105 (1990); C. Pike, A. Walencewitz, C. Glabe, C. Cotman, *Brain Res.* **563**, 311 (1991).
22. J. Baudier and R. D. Cole, *J. Biol. Chem.* **262**, 17577 (1987).
23. C. M. van Duijn *et al.*, *Lancet* **337**, 978 (1991).
24. J. A. Mortimer *et al.*, *Int. J. Epidemiol.* **20 Suppl. 2, S28** (1991).
25. G. W. Roberts, *Lancet* ii, 1456 (1988); G. W. Roberts, D. Allsop, C. Bruton, *J. Neurol. Neurosurg. Psychiatry* **53**, 373 (1990).
26. T. Kawarabayashi, M. Shoji, Y. Harigaya, H. Yamaguchi, S. Hirai, *Brain Res.* **563**, 334 (1991); R. Siman, J. P. Card, R. B. Nelson, C. W. Cotman, *Neuron* **3**, 275 (1989); G. W. Roberts, S. M. Gentleman, A. Lynch, D. I. Graham, *Lancet* **338**, 1422 (1991).
27. D. Goldgaber *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7606 (1989).
28. K. Abe, P. H. St. George-Hyslop, R. E. Tanzi, K. Kogure, *Neurosci. Lett.* **125**, 169 (1991); K. Abe, R. E. Tanzi, K. Kogure, *ibid.*, p. 172.

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