Alzheimer’s disease: dysfunction of a signalling pathway mediated by the amyloid precursor protein?

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Abstract

All individuals with Alzheimer’s disease (AD) experience a progressive loss of cognitive function, resulting from a neurodegenerative process characterized by the deposition of β-amyloid (Aβ) in plaques and in the cerebrovasculature, and by the formation of neurofibrillary tangles in neurons. The cause of the neuronal death is unknown but it is thought to be linked in some way to the β-amyloid precursor protein (APP), which is the source of the Aβ that accumulates in the AD brain. There are two pieces of supporting data for this: first, APP is overexpressed in Down’s syndrome, which leads to AD-like neuropathology by the age of 40 in virtually all affected individuals; secondly, specific point mutations in APP cause some forms of familial AD. Our laboratory has focused on a specific aspect of APP and its connection with the neuronal destruction seen in AD. We have hypothesized that AD results from a progressive dysfunction of APP. In addition, on the basis of recent data generated by our laboratory and others, we propose that in the normal brain a percentage of APP is present as an integral protein of the plasma membrane that mediates the transduction of extracellular signals into the cell via its Aβ-containing C-terminal tail. In AD, accumulation of abnormal levels of the C-terminus in the neuron disturbs this signal-transduction function of APP, causing disorders in the cell-cycle machinery and consequent apoptosis. Here, we discuss the key findings that support this hypothesis, and discuss its therapeutic implications for AD.

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Alzheimer’s disease (AD) is characterized by a progressive loss of cognitive function, resulting from a neurodegenerative process characterized classically by the deposition of β-amyloid (Aβ) in plaques and in the cerebrovascularity, and the formation of neurofibrillary tangles in neurons. Additional pathological hallmarks of AD include granulovacular degeneration, loss of synapses and decreases in cell density in distinct regions of the brain. AD does not have a simple aetiology. It can occur as a ‘sporadic’ event, it can result from the possession of an extra copy of chromosome 21 (Down’s syndrome), or it can be caused by mutations in the β-amyloid precursor protein (APP) gene on chromosome 21 or in the presenilin genes on chromosomes 1 and 14. Additional genetic complexity is conferred on it by the fact that the ε4 allele of the APOE gene is a major risk factor for the development of AD. Thus it is unlikely that AD is caused by a single molecular event.

Numerous mechanisms for the neuronal cell death in AD have been proposed. One of these is the amyloid hypothesis, which suggests that deposition of Aβ is a primary event in the pathological cascade for AD. This argument is based on studies in vitro showing that Aβ is toxic to neurons and on the measurement of the increase in release of Aβ by cells carrying familial AD (FAD) mutant genes.

There are two major C-terminal variants of Aβ: Aβ40 is the major species secreted from cultured cells and is found in cerebrospinal fluid, whereas Aβ42 is the major component of amyloid deposits [1]. Cells expressing FAD mutants of APP and the presenilins are reported to secrete increased amounts of Aβ42, suggesting a link of this variant of Aβ to AD pathogenesis. Consequently, a leading hypothesis for the aetiology of AD is that increased Aβ42 is a shared molecular correlate of FAD mutations, and that it represents a gain of deleterious function that can cause FAD [2] and may be an essential-early event in AD [1]. Although this ‘amyloid hypothesis’ is attractive, molecular mechanisms other than those mediated by extracellular Aβ could also lead to AD neurodegeneration.

These mechanisms are likely to be linked in some way to APP, the source of Aβ. One of the most compelling pieces of evidence that links AD neurodegeneration to APP and/or its Aβ-containing derivatives is the early finding that the APP gene is on chromosome 21: virtually all individuals trisomic for this chromosome show AD-like neuropathology by 40 years of age. Additionally, it has been discovered that specific mutations in APP cause some forms of familial FAD. These data have raised the possibility that AD may result from an alteration in the normal function of APP [3,4], and have re-focused attention on the delineation of the function that APP subserves in the brain. It has been shown [5,6] that in the brain a percentage of APP is present on the cell surface, and it is proposed [4,6] that this cell-surface APP mediates the transduction of extracellular signals into the cell via its C-terminal tail.

Nishimoto and colleagues [7] showed that APP binds to the brain-specific, signal-transducing G-protein, G_{i3}; independent confirmation of this
finding has subsequently been published [8,9]. It was then discovered [10] that Val642 (‘London’) FAD mutants of APP induce neuronal DNA fragmentation, a feature of apoptosis, in a neuronal cell line. This fragmentation is independent of Aβ42 production [10] and is mediated by the Gβγ complex of Go [11]. These data support the notion that APP has an intrinsic signalling function in the neuron, which becomes ligand independent when APP is mutated at Val642.

To examine the mechanism by which FAD APP might cause apoptosis in neurons, we expressed five different Alzheimer mutations of APP in primary neurons via recombinant herpes simplex virus (HSV) vectors, and quantified the levels of APP metabolites [12]. The predominant intracellular accumulation product was a C-terminal fragment of APP that co-migrated with the protein product of an HSV recombinant expressing the C-terminal 100 amino acids (C-100) of APP. Interestingly, we had proposed previously that C-100 is involved in the aetiology of AD [13]. It is neurotoxic in vitro [14–18] and is amyloidogenic [19–25]. In addition, expression of C-100 in vivo can cause neuropathology that is similar in some ways to that in AD, including neurodegeneration and cognitive dysfunction [26–33], as well as increases in acetylcholinesterase [34] and abnormalities in synaptic transmission [35]. There has been some question of whether C-100 exerts its neurotoxic effects from inside or outside the cell [36,37]. Our data of the past 6 years suggest strongly that C-100 kills from inside the cell; this is supported by the observation that C-100 is not secreted, even when it carries a signal peptide [12,22,38]. Although at least one group has reported neurotoxicity resulting from the addition of C-100 to the culture medium [39], we believe that this type of neurotoxicity is mechanistically different from the neurodegeneration caused by the expression of C-100 within neurons.

The fact that APP interacts with the signalling molecule Go, that FAD mutants of APP can cause Go-mediated apoptosis in neuronal cells and that the same FAD mutants of APP cause the intracellular accumulation of C-100 suggested to us the following working hypothesis: in the brain a portion of APP is present as an integral plasma membrane protein that mediates the transduction of extracellular signals into the cell via its C-terminal tail; abnormal accumulation of its Aβ-containing C-terminus in the neuron causes progressive dysfunction of APP signalling in AD, resulting in apoptosis. This hypothesis was further supported by the finding that the intracellular C-terminal tail of APP also interacts with the protein APP-BP1 [40], and with members of the Fe65 family of adaptor proteins [41].

### Processing of APP

Most of what is known about APP processing has come from work with cultured cells. APP matures through the constitutive secretory pathway. Some of the APP is endoproteolytically cleaved at the cell surface within the Aβ sequence by the α-secretase, which generates the neuroprotective, secreted APP and non-amyloidogenic, 3 kDa Aβ-secreted products [42–44]. Secreted APP is readily detected in human plasma and cerebrospinal fluid.
Endocytosis of cell-surface APP generates C-terminal fragments of 8–12 kDa that are degraded in the lysosomes [45,46]; some of these, generated by β-secretase cleavage at the N-terminus of the Aβ sequence, are analogous to C-100 and are amyloidogenic. Activity of a second protease, γ-secretase, cleaves these C-terminal fragments of APP to release the full-length Aβ protein. Thus both Aβ and C-100-like fragments of APP are produced normally at low levels by the cell. Most of the Aβ that is generated is secreted, although small amounts can be detected intracellularly. C-100-like amyloidogenic fragments have only been detected intracellularly.

It is important to note that APP processing is cell-type specific. LeBlanc and colleagues have reported that human neurons secrete more 4 kDa Aβ than 3 kDa Aβ, and metabolize approximately 40% of newly synthesized APP through the α-secretase pathway [47,48]. Moreover, human neurons produce five C-terminal fragments of APP, in a pattern seen uniquely in the human brain [47,49]. The two largest C-terminal derivatives have the entire Aβ sequence at or near their N-terminus [49], and probably represent endogenous C-100 fragments. Thus C-100 is a physiologically relevant fragment of APP in the human brain. In contrast with human neurons, most APP-transfected human or non-human cell lines produce more 3 kDa Aβ than 4 kDa Aβ, and show a relatively non-amyloidogenic pattern of C-terminal fragments [45,46,50,51].

Analyses of Aβ in genetically engineered cell lines expressing FAD mutations in both APP and the presenilins have shown that all of the mutations cause either increased overall secretion of Aβ or increased secretion of the long (42–43-amino-acid) form of Aβ (Aβ42) relative to the shorter 40-amino-acid form [2]. Increases in Aβ42 have also been detected in transgenic mice expressing FAD mutations of both APP and presenilin (PS) [2]. Aβ42 is the major component of brain amyloid deposits in AD. Consequently, a leading hypothesis for the etiology of AD is that increased Aβ42 is a shared molecular correlate of FAD mutations which may also be operative in ‘sporadic’ AD. Increases in Aβ42 have not been shown directly in human AD brain homogenates, although it is clear that amyloid plaques contain a disproportionate amount of Aβ42. Furthermore, analyses of levels of this peptide in the plasma and cerebrospinal fluid of AD patients have revealed no differences between AD patients and controls in the plasma [52], and a reduction of Aβ42 in the cerebrospinal fluid of AD patients relative to controls [52,53]. However, increased release of Aβ42 from fibroblasts of AD patients with mutations in the presenilins, as well as increased levels of Aβ42 in their plasma, has been demonstrated [54].

The β-secretase cleavage product of APP, C-100, is increased in cell lines expressing the Swedish FAD mutation of APP [55,56], but not in cell lines expressing the London Val642 mutation of APP [57]. Since neurons process APP differently from cell lines, we expressed all known FAD mutants of APP in primary neurons in culture, and analysed APP processing in the infected neurons. We showed that all the mutants caused increases in the intracellular levels of C-100 [12]. We have not yet determined whether FAD mutations in the presenilins cause the same alterations in C-100 levels. Although increases of
C-100 in FAD APP transgenic animal models have not been published, at least one such model, expressing the Val$^{642}$→Phe (V642F) mutation of APP (numbered according to the APP$_{695}$ of APP) [58], shows a significant increase in C-100 in brain homogenates. We have hypothesized that abnormal accumulation of C-100 in the neuron occurs in AD; however, it has not yet been shown whether the level of C-100 protein is increased in human AD versus control brains post mortem.

Even if C-100 levels are increased in AD brains, this does not prove that C-100 causes the neuropathology of AD. One issue is whether the neurodegeneration is caused by intact C-100 or by the Aβ that is generated from C-100. To answer this question, it will be necessary to create mutants of C-100 and of FAD APPs that inhibit γ-secretase cleavage and production of Aβ [59], and determine whether these mutants cause neurodegeneration in vitro or AD-like pathology in vivo. This leads us to the question of whether the apoptosis caused by FAD mutants of APP in neuronal cells [10] is due to the increased intracellular C-100 that results from expression of these mutants. To answer this, it will be necessary to mutate the β-secretase cleavage site in the London FAD mutant of APP, show that it inhibits the production of C-100, and assay this mutant relative to the non-mutated London FAD mutant for its ability to cause apoptosis in neurons. If inhibition of γ-secretase cleavage of C-100 increases the accumulation of C-100 in the neurons, and does not inhibit apoptosis caused by C-100 or by the FAD APP mutants, the safety of therapeutic agents that inhibit γ-secretase is called into question.

### APP as a signalling molecule

The possibility that APP may act as a signalling receptor was first proposed on the basis of its predicted amino acid sequence, which suggested that APP was a type-1 intrinsic membrane protein consistent with the structure of a cell-surface receptor [60]. However, subsequent studies of the function of APP concentrated largely on the secreted ectodomain, because of a lack of direct evidence that mature APP exists on the cell surface with intact intracellular, transmembrane and extracellular domains. APP was inferred to exist on the surface of a variety of cultured cells [46,61,62], but some laboratories could not detect it [63]. Nevertheless, some reports demonstrating involvement of APP in neuronal development, synaptogenesis and synaptic plasticity [64–69] did not restrict the observed function to secreted APP, raising the possibility that some aspects of synaptic plasticity are mediated by cell-associated APP. Indeed, it has now been demonstrated directly that some APP is found on the cell surface in neurons [5,6,70]. Cell-surface APP possesses a neurite-promoting activity that is distinct from that of the secreted APP [69], co-localizes with adhesion plaque components [70,71] and participates in synaptic vesicle recycling [72], suggesting that a percentage of APP may function as a cell-surface receptor, transducing signals from the extracellular matrix to the interior of the cell (Figure 1).

The growth cone G-protein, Gs [7], the presumptive adaptor proteins Fe65 and X11 [41], and APP-BP1 [40] have been reported to interact with the
C-terminus of APP, presumably to initiate intracellular signalling (Figure 1). Although the functions of Fe65 and X11 are not known, Fe65 has the characteristics of adaptor proteins, which are thought to link signal-transduction events emanating from plasma membrane receptors to intracellular molecules by forming complexes of these proteins. Therefore, one could envisage APP as being part of a $G_\alpha$-protein-centred complex that transduces extracellular signals to the cytoplasm and the nucleus, with Fe65 linking APP to molecules downstream in the pathway. APP-BP1 could be one of the downstream molecules.

The molecule whose interaction with APP has been defined in greatest detail is $G_\alpha$. Nishimoto and colleagues have demonstrated that the His$^{657}$–Lys$^{676}$ domain of the APP$\text{}_{695}$ isoform activates the heterotrimeric $G_\alpha$ in a GTP$^\gamma$/H9253-inhibitable manner [7,73]. Their demonstration that an antibody to the extracellular domain of APP that acts as a ligand mimetic [74] causes activation of $G_\alpha$, provides an argument for APP being a G-protein-coupled receptor. As noted earlier, the London mutation of APP, V642I, causes DNA fragmentation when expressed in a neuronal cell line [10]. Notably, expression in the cells of V642I APP deleted for residues His$^{657}$–Lys$^{676}$ did not cause DNA fragmentation. Pertussis toxin, an inhibitor of $G_\alpha$ and $G_\beta$, blocked the DNA fragmentation caused by V642I, as did co-transfection of V642I APP and a cDNA encoding a dominant-negative mutant of $G_\alpha$, but not with a cDNA encoding a dominant-negative mutant of $G_\beta$. Inhibition of A$\beta$42 production from the V642I APP by mutating the $\gamma$-secretase cleavage site did not have any effect on the DNA fragmentation caused by V642I.

These data suggest that $G_\alpha$ mediates the DNA fragmentation caused by the V642I mutants of APP; indeed, a subsequent study by Nishimoto’s group revealed that the DNA fragmentation was mediated by the $\beta\gamma$ complex of $G_\alpha$ [11]. A$\beta$ does not appear to play a causative role in inducing DNA fragmentation in this experimental paradigm. The data support the notion that these
mutants act by causing unregulated activation of Go and, by inference, of a cellular signalling pathway downstream of Go.

**Apoptosis and the cell cycle in AD**

The notion that a form of cell suicide called apoptosis participates in the neuropathology of AD was raised by Su et al. [75], when they reported evidence for DNA fragmentation in neurons in the AD brain. Although other groups have also detected this feature of apoptosis in the AD brain, many in the field have been sceptical of the idea that the neurons that die in AD undergo apoptosis, partly because DNA fragmentation can also be caused by oxidative damage [76] or by autolysis post mortem [77]. However, a recent report from the laboratory of Mark Mattson [78] has revived interest in the possibility that apoptosis is operative in AD. These investigators found that levels of a marker of apoptosis, prostate apoptosis response-4 protein (Par-4), are increased 15–20-fold in vulnerable neurons in AD brain compared with controls. They also showed that Par-4 expression is increased in cultured neurons undergoing apoptosis, and that inhibition of Par-4 expression in these neurons blocks apoptosis.

These findings provide the strongest evidence to date that neuronal death in AD may be due to apoptosis, and they are consistent with the increasing awareness in the field that at least one of the normal functions of APP is to regulate apoptosis in the neuron. As noted earlier, Nishimoto’s group showed that the V642I mutation of APP causes DNA fragmentation when expressed in a neuronal cell line [10]. Zhao and co-workers [79] showed that the same mutation, as well as two additional FAD APP mutations, induced apoptosis in differentiated PC12 cells. Barnes and colleagues [80] reported that levels of APP are increased in motor neurons dying of apoptosis, and that APP is cleaved by caspase-3, a caspase activated in apoptotic motor neurons. Interestingly, we [81] and others [82] have shown that overexpression of wild-type APP causes apoptotic death of neurons, although to a lesser degree than does expression of FAD mutants of APP.

Approximately 50% of inherited AD cases are caused by mutations in the presenilin genes PS1 and PS2. It has been reported that overexpression of these genes in transfected cell lines can cause apoptosis [83] or result in an increased susceptibility to apoptosis [78, 84–86]. On the other hand, we have found [81] that expression of PS1 in primary neurons does not cause or enhance apoptosis; rather, it protects neurons against experimentally induced apoptosis. Thus the ability of PS1 to induce apoptosis appears to be cell-type specific; and this may have important implications for the pathogenesis in AD, in which neurons are differentially affected. PS1 is reported to be expressed primarily in central nervous system neurons in the brain, suggesting that this protein may perform a neuron-specific function [87]. In fact, in AD, neurons that express PS1 antigen are less vulnerable to the disease than neurons that do not express it [88], and inhibition of PS1 expression results in apoptosis [89], suggesting a protective role for this protein. Although the precise role of presenilins in regulation of apoptosis in the neuron is still unclear, the evidence that they do play a role in
this pathway is strong. These data implicate both APP and presenilins in the control of apoptotic death in the brain, and it is not unreasonable to suppose that FAD mutations in these genes may cause dysfunction in this pathway.

The implication of apoptosis in AD aetiology is consistent with the numerous findings of cell-cycle abnormalities in AD. Apoptosis and the cell cycle are closely coupled, and the re-expression of cell-cycle markers has been linked with the occurrence of certain types of neuronal cell death [90–92]. The interpretation of these findings [92] is that a neuron is committed to the permanent cessation of cell division, so if, for any reason, it is forced to re-enter the cell cycle after this commitment, it dies. Notably, ectopic expression of cell-cycle proteins and their associated kinases in the AD brain has been reported [94–97]. Most recently, Busser and co-workers [98] found abnormal appearance of cell-cycle markers in regions of the AD brain where cell death is extensive; in addition, Chow and colleagues [99] found increases in the expression of genes encoding cell-cycle proteins in single neurons in late-stage relative to early-stage AD brain. The phosphoepitope Ser214 of the microtubule-associated protein tau, which appears in the neurofibrillary tangles in AD, is a prominent phosphorylation site in metaphase, but not in interphase, of dividing cells expressing tau [100]. This supports the view that reactivation of the cell-cycle machinery may be involved in hyperphosphorylation of tau in AD brain. The possibility that phosphorylation-dependent events occurring during the cell cycle affect the normal function of APP is suggested by the finding that regulation of the phosphorylation and metabolism of this protein occurs in a cell-cycle-dependent manner [101,102].

We hypothesize that dysfunction of pathways mediated by APP may be one cause of the reactivation of cell-cycle proteins in the AD brain. It is important to increase our understanding of the normal function of APP in the brain, identify which fragment(s) of APP causes apoptotic neuronal death and clarify some of the molecular events that lead to this apoptosis. Given the recent evidence that apoptosis and cell-cycle abnormalities occur in the AD brain, the data that are obtained may suggest a mechanism by which neurons die in AD.

References


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