Alzheimer’s disease: inside, outside, upside down

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Abstract

Neurotoxicity of β-amyloid peptide (Aβ) in Alzheimer’s disease (AD) is usually thought to arise from the nonspecific effects of high concentrations of Aβ on vulnerable neurons, resulting in membrane destabilization and increasing intracellular calcium concentration. This review advances the hypothesis that at early stages of AD, when Aβ is present in lower amounts, its ability to perturb the function of cellular targets is mediated by specific cofactors present on the cell surface and intracellularly. Receptor for advanced glycation endproducts (RAGE) is a cell-surface receptor which binds Aβ and amplifies its effects on cells in the nanomolar range. The intracellular enzyme Aβ-binding alcohol dehydrogenase (ABAD) is likely to engage nascent Aβ formed in the endoplasmic reticulum, and to mediate cell stress from this site. The analysis of Aβ interaction with RAGE and ABAD, as well as other cofactors, provides insight into new mechanisms and, potentially, identifies therapeutic targets relevant to neuronal dysfunction in AD.

Introduction

Extracellular accumulations of amyloid in neuritic plaques, composed predominately of β-amyloid (Aβ) peptide and intracellular neurofibrillary tangles, composed in large part of hyperphosphorylated paired helical filament tau, are pathognomonic features of Alzheimer’s disease (AD) [1–5]. With time, these lesions increase in number and volume, concomitant with neuronal toxicity and death [1–6]. While amyloidogenic peptides are condensed into copious fibrillar deposits in the middle to late stages of AD, the relationship of such lesions to mechanisms of early cellular dysfunction is undefined. It is possible that the densely packed deposits of Aβ found at the centre of neuritic plaques

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may have a protective function by sequestering toxic amyloid from cellular elements.

**Aβ interaction with cell surface receptors**

In AD it is widely accepted that later in the course of the disease, when Aβ fibrils are abundant, non-specific interactions of such fibrils with the cell surface may be frequent and disruptive for cellular functions [7-12] (Figure 1, left). Aβ fibrils can destroy plasma membranes, causing changes in ionic homoeostasis, and could trigger cell death by several mechanisms. However, earlier in the course of the disease when Aβ fibrils are present at lower levels, and Aβ is presumably present principally in oligomeric soluble forms, higher affinity interactions with cellular surface molecules are more likely to be relevant (Figure 1, right). One such molecule is the immunoglobulin superfamily receptor RAGE (receptor for advanced glycation endproducts). In the AD brain, this receptor is expressed at higher levels in neurons and microglia proximate to neuritic plaques and in cells of Aβ-loaded blood vessels, than in those areas free of pathology or in control brains [13]. RAGE binds Aβ in its soluble monomeric/oligomeric and insoluble fibrillar forms with nanomolar affinity, targeting the amyloidogenic peptide to the cell surface. In culture, cells expressing RAGE are more susceptible to Aβ-induced cellular dysfunction than those with lower levels of RAGE, or those in which the receptor is blocked.

**Figure 1** Schematic depiction of non-specific (left) and specific (right) interactions of Aβ with cellular elements. Aβ monomer/oligomers/fibrils and cell binding proteins for Aβ (Y) are shown in relation to the cell membrane. Resulting increases in cytosolic free calcium [Ca], and reactive oxygen intermediates (ROI) are also noted. Reprinted from Biochim. Biophys. Acta, **1502**, Yan, S.D., Roher, A., Chaney, M., Zlokovic, B., Schmidt, A.M. and Stern, D., Cellular cofactors potentiating induction of stress and cytotoxicity by amyloid beta-peptide, 145–157. © (2000) with permission from Elsevier Science.
Consistent with a role for Aβ-receptor interactions in the early disruption of neuronal functions, are the findings that Aβ binding to neuronal RAGE results in activation of nuclear factor κB (NF-κB), induction of haem oxygenase type 1 and expression of macrophage-colony stimulating factor (M-CSF), each of which can be demonstrated in AD brain [13,14]. In contrast, cells expressing low levels of RAGE require greater concentrations of Aβ to bring about changes in cellular properties. Cellular disruption resulting from low levels of Aβ binding to RAGE reflects active redirection of cellular biosynthetic mechanisms, not non-specific and rapid induction of cell death, which is observed at high levels of Aβ (the latter is independent of RAGE). Therefore, Aβ–RAGE interaction could contribute to the well-recognized chronic inflammatory component underlying AD. Aβ stimulation of RAGE causes nuclear translocation of NF-κB, thereby increasing the expression of M-CSF by neurons (which also spills over into cerebrospinal fluid) and resulting in the recruitment and activation of microglia to early sites of cellular disruption [14] (Figure 2).

In view of the multiple conformational structures into which Aβ assembles, it is to be expected that the amyloidogenic peptide will interact with several cell-surface recognition sites, potentially opportunistic receptors whose activation could engender varied alterations in cellular phenotype. Consistent with this theory, the macrophage scavenger receptor, expressed selectively in the brain on microglia, has been shown to bind and internalize Aβ fibrils [15,16]. Such endocytosis of Aβ could enhance a protective function, by sequestering and disposing of toxic peptides, or alternatively could contribute to pathogenicity by promoting chronic cellular dysfunction. In this context, increased expression of megalin/gp330, a lipoprotein receptor that serves as an acceptor site for apolipoprotein E (ApoE) [17], in neurons that display intracellular ApoE and Aβ as well as evidence of DNA fragmentation suggests that

uptake of the ApoE/Aβ complex via this receptor system may load cells with intracellular Aβ in a relatively stable complex that promotes toxicity, and eventually apoptosis [17]. Although cell-surface RAGE [18], and presumably other Aβ cellular binding sites, are not necessary or even sufficient mediators of Aβ-induced cytotoxicity (which can occur in the absence of RAGE given a sufficiently high concentration of Aβ), the presence of such receptors may serve to focus and amplify the toxic effects of Aβ, especially at relatively low amounts of peptide and when Aβ is in filamentous form. If only by sustained approximation of Aβ to the cell surface, RAGE can effectively increase toxicity but, in addition, binding of Aβ to RAGE may trigger intracellular signals deleterious to cell function and viability. Finally, RAGE can also serve to assist clearance of very small amounts of Aβ (or filaments) through interaction of the Aβ–RAGE complex. The ability of RAGE to bind Aβ and advanced glycation endproducts (AGEs) [19] is in all likelihood an accident of evolution due to molecular mimicry with other RAGE ligands such as amphoterin [20], which promotes neurite outgrowth in the developing brain. A result of the proposed involvement of Aβ receptors in the pathogenesis of AD is that early in the course of this disorder, before irreversible cellular events occur, inhibition of Aβ–cell-surface interactions could be neuroprotective, and potentially therapeutic. However, this hypothesis must await development of appropriate transgenic animal models that imitate the pathophysiology of AD to be proved completely. In this context, mice overexpressing RAGE and/or the scavenger receptor, along with other susceptibility factors such as presenilins or β-amyloid precursor protein (APP) mutants, may represent excellent candidates for such models.

**Aβ interaction with the intracellular enzyme ABAD (Aβ-binding alcohol dehydrogenase)**

Evidence linking increased production of Aβ, in culture and in the brains of transgenic mice that are overexpressing presenilins or which have mutations in APP, with early-onset familial AD have highlighted a role for Aβ in the pathogenesis of neurodegeneration [1-3,21–24]. Processing of APP may occur by several pathways [1], including generation of the amyloidogenic peptide within the endoplasmic reticulum [25–28], also a site for localization of presenilin [29]. Immunoprecipitation of APP–presenilin complexes formed within the cell indicates that these two molecules may interact *in vivo*, potentially affecting the processing of APP [30]. Using the yeast two-hybrid system, we have identified a polypeptide, ABAD, which specifically binds Aβ. ABAD was found in the endoplasmic reticulum and in mitochondria and was originally named ERAB (endoplasmic reticulum-associated Aβ binding protein), after its first site of intracellular localization within the endoplasmic reticulum. However, it is now referred to as ABAD in view of its functional properties and presence in multiple subcellular compartments [31]. ABAD binds with nanomolar affinity to the Aβ monomer/oligomer, as well as to Aβ assembled into fibrils, and it potentiates Aβ-induced cellular toxicity, assessed by induction of apoptosis. The blockage of ABAD–Aβ interaction, through the intro-
duction of anti-ABAD F(ab')2 into cells, using liposomes, suppressed Aβ-induced apoptosis at lower concentrations of amyloidogenic peptide (up to 1 μM). However, at higher concentrations of Aβ (< 10 μM), the effects of ABAD were not discernible, presumably due to an excess of non-specific Aβ–cellular interactions. Immunohistochemical analysis of the AD brain shows ABAD at low levels in normal cortical neurons, but with substantially increased expression in AD neurons in the brains of patients with AD. ABAD is an intriguing cellular target of Aβ as it is the human counterpart of type II 3-hydroxyacyl-CoA dehydrogenase, an enzyme which participates in the third reaction of the β-oxidation spiral [32], and is therefore integral to cellular energy and fatty acid metabolism. Based on protein sequence homologies, ABAD could also be a short chain-alcohol dehydrogenase, which suggests its potential to generate toxic aldehydes within disrupted cells. Of course, the discovery of ABAD raises many questions with respect to how such an intracellular polypeptide gains access to Aβ, whether ABAD enzymic activity is related to its potential role in cellular toxicity, and, if this is not the case, whether its activity is modulated by Aβ. These considerations indicate the extent to which further studies will be required to determine a possible role for ABAD in Aβ-mediated neurotoxicity. In this context, it is possible that intracellular Aβ, concentrated in cellular compartments such as the endoplasmic reticulum and Golgi or in the endosomal–lysosomal pathway, damages cell membranes and gains access to otherwise remote subcellular targets [33]. Other neurotoxic species also interact with enzymes fundamental in cellular metabolism, as illustrated by the binding of huntingtin and other proteins containing polyglutamine domains to glyceraldehyde-3-phosphate dehydrogenase [34–36]. The potential of Aβ, an enzyme that can participate in cellular homoeostasis, to modulate properties of an intracellular target (Figure 3) opens a new view to
possible early cellular events signalling trouble within neurons at a time when intervention might reverse early functional perturbations.

Conclusion

There is still much unknown about the pathogenesis and even pathological manifestations of sporadic AD. This is exemplified by the recent recognition of distinct hitherto unsuspected extracellular proteinaceous deposits of ~100 kDa polypeptide in AD with monoclonal antibodies to plaque-derived substances [37]. The identification and characterization of specific cellular targets of Aβ, such as RAGE, scavenger receptor, megalin/gp330 and ABAD provides a context for considering early cellular perturbations due to Aβ, in relation to both cell-surface binding sites/receptors and intracellular targets, and may provide new insights into molecular mechanisms and future therapeutic approaches.

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References


