The role of cell-derived oligomers of Aβ in Alzheimer’s disease and avenues for therapeutic intervention

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Abstract
Burgeoning evidence suggests that soluble oligomers of Aβ (amyloid β-peptide) are the earliest effectors of synaptic compromise in Alzheimer’s disease. Whereas most other investigators have employed synthetic Aβ peptides, we have taken advantage of a β-amyloid precursor protein-overexpressing cell line (referred to as 7PA2) that secretes sub-nanomolar levels of low-n oligomers of Aβ. These are composed of heterogeneous Aβ peptides that migrate on SDS/PAGE as dimers, trimers and tetramers. When injected into the lateral ventricle of rats in vivo, these soluble oligomers inhibit hippocampal long-term potentiation and alter the memory of a complex learned behaviour. Biochemical manipulation of 7PA2 medium including immunodepletion with Aβ-specific antibodies and fractionation by size-exclusion chromatography allowed us to unambiguously attribute these effects to low-n oligomers. Using this paradigm we have tested compounds directed at three prominent amyloid-based therapeutic targets: inhibition of the secretases responsible for Aβ production, inhibition of Aβ aggregation and immunization against Aβ. In each case, compounds capable of reducing oligomer production or antibodies that avidly bind Aβ oligomers also ameliorate the synaptotoxic effects of these natural, cell-derived oligomers.

Introduction
Alzheimer’s disease (AD) is the most common human dementia. In the year 2000, there were an estimated 4.5 million persons with AD in the U.S.A., and in the absence of effective therapy, this number is set to triple by 2050 [1]. The precise onset of clinical AD is very difficult to discern, with mild memory impairment the earliest symptom, but as the disease progresses other cognitive and behavioural changes accrue [2,3]. The end-stage AD brain is characterized by atrophy of the hippocampal formation and cerebral cortex and ventricular enlargement. Microscopically, amyloid plaques and NFTs (neurofibrillary tangles) are detected throughout the hippocampus and cerebral cortex and are often accompanied by variable numbers of amyloid-bearing meningeal and cortical microvessels. The principal component of NFTs is the microtubule-associated protein, tau. Plaques and vascular deposits are chiefly composed of Aβ (amyloid β-proteins) that are generated by sequential proteolysis of the APP (β-amyloid precursor protein) by enzymes known as β- and γ-secretase [4–6] (Figure 1). Although many studies have sought to correlate the severity of dementia with the number of amyloid plaques or NFTs, the best statistical correlations exist between measures of synaptic density and degree of dementia [7–9]. In fact, the decrease in synapse number and density is disproportionate to the loss of neuronal cell bodies [7,10,11] and suggests that pruning of processes occurs prior to neuronal death.

The emerging role of soluble Aβ
Diverse lines of evidence suggest that Aβ plays a central role in the pathogenesis of neuronal dysfunction in AD [12–14], yet the Aβ hypothesis remains controversial, not least because the quantity and temporal progression of amyloid plaques do not show a simple relationship to the clinical progression of the disease [15]. However, recent studies suggest that the relatively weak correlation between plaque burden and severity of cognitive impairment may be explained by the activity of multiple different Aβ assembly forms and that early memory impairment may be mediated by soluble low-n oligomers. To model Aβ-mediated neurotoxicity, many investigators have used synthetic peptides (for a review, see [16]). At ambient or body temperature and at concentrations ≥10–20 µM, both synthetic Aβ1-40 and Aβ1-42 self-associate to form low-n oligomers, PFs (protofibrils) and fibrils (Figure 1). An important caveat when considering
the cellular effects of different A\(\beta\) assemblies is the highly dynamic nature of A\(\beta\) aggregation. Because intermediates can further associate into higher-ordered aggregates, it is difficult to unambiguously ascribe cytopathological activity to a discrete species. Nonetheless, several groups have attempted to isolate pre-fibrillar synthetic A\(\beta\) assemblies and probe their toxic activity. In 1998, Lambert et al. [17] presented the first experimental evidence that certain soluble, non-fibrillar assemblies of synthetic A\(\beta\) [which they called ADDLs (A\(\beta\)-derived diffusible ligands)] could be neurotoxic. ADDLs are only formed under certain specific conditions, but once formed they are relatively stable [17,18]. By atomic force microscopy ADDLs appear as spheres with a diameter of approx. 5 nm, and migrate on SDS/PAGE at approx. 4, 8, 16 and 18 kDa. ADDLs have been shown to cause neuronal death in culture, block LTP (long-term potentiation) [17,19] and inhibit reduction of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2\(H\)-tetrazolium bromide] by neural cell lines [17,18]. When incubated with organotypic mouse brain slices at 500 nM for 45–60 min, cell loss was not evident but a near-complete block of LTP was observed [17,19]. It is conceivable that during their incubation with neurons, ADDLs may form larger A\(\beta\) assemblies; however, the electrophysiological experiments were performed over a short-time course (1–2 h) and at concentrations (~500 nM) well below the critical concentration for synthetic A\(\beta\) fibril formation in vitro, suggesting that ADDLs are themselves synaptotoxic.

Another non-fibrillar assembly, PF, can also rapidly alter neuronal function. PF range from spherical assemblies of approx. 5 nm diameter to short, flexible rods of up to 200 nm in length [20,21], but unlike, ADDLs, PF can be generated under a variety of biochemical conditions, and their rate of formation is dependent on A\(\beta\) concentration, pH and ionic strength [22]. PF appear to behave as true fibril intermediates in that they can both form fibrils and dissociate to lower-molecular-mass species [22,23]. Using whole-cell patch-clamp recordings, PF composed of A\(\beta\)1-40 induced an instantaneous increase in EPSCs (excitatory post-synaptic currents) in rat cortical neurons [24]. Fibril preparations also enhanced EPSCs, whereas monomeric A\(\beta\) had no effect. This excitability was entirely reversible and was concentration-dependent, with activity starting at low micromolar concentrations. Moreover, PF appear to have inherent electrophysiological activities distinct from fibrils [25] suggesting that PF and fibrils may act by separate mechanisms.

Cell-derived oligomers of A\(\beta\) disrupt both synaptic plasticity and learned behaviour

While there is no doubt that soluble pre-fibrillar assemblies of synthetic A\(\beta\) can alter synaptic function, there is as yet no confirmation that these species actually occur in nature. Thus instead of studying the activity of A\(\beta\) assemblies generated from synthetic peptides, we chose to study the activity of naturally produced, cell-derived A\(\beta\) oligomers. SDS-stable oligomers (~8 and 12 kDa) of A\(\beta\) have been detected in the buffer-soluble fraction of human cerebral cortex [26], in human cerebrospinal fluid [27,28] and in a variety of cultured cells [28–31]. 7PA2 cells (CHO (Chinese-hamster ovary) cells that express a mutant V717F (Val717 \(\rightarrow\) Phe) human APP) produce and secrete significant amounts of SDS-stable A\(\beta\)
low-\(n\) oligomers [29] that migrate in denaturing gels as dimers, trimers and occasionally tetramers [32]. Importantly, the species detected in 7PA2 CM (conditioned medium) has been confirmed as bona fide \(A\beta\) oligomer by both N-terminal radiosequencing and precipitation with \(A\beta_{42}\) - and \(A\beta_{42}\)-specific C-terminal antibodies [28,29]. Because of the easy maintenance and fast growth rate of 7PA2 cells, 7PA2 CM has been our medium of choice to investigate the biological activities of cell-derived \(A\beta\) oligomers. Microinjection of small volumes (\(\sim 1.5\ \mu l\)) of such 7PA2 CM into the lateral ventricle of the brain of a live rat inhibited hippocampal LTP [33]. Evidence that the blockage of LTP was mediated by \(A\beta\) oligomers emerged from biochemical manipulation of the sample. Immunodepletion of the CM with \(A\beta\)-specific antibodies prevented the blockage of LTP, whereas immunodepletion of the abundant soluble APP-\(\alpha\) derivative had no effect. Most importantly, preincubation of the CM with insulin degrading enzyme, a protease that efficiently deggrades \(A\beta\) could render the CM inactive, and that SEC fractions containing fractions had no effect [38]. Independent support for our finding that dimers and trimers of \(A\beta\) can interfere with the memory of a learned behaviour, comes from the report that the appearance of dimeric \(A\beta\) in cortical lipid raft fractions coincides with the first indicators of behavioural compromise in APP transgenic mice [39].

### Avenues for therapeutic intervention

The data reviewed above clearly demonstrate that cell-derived oligomers of \(A\beta\) disrupt both synaptic plasticity and learned behaviour in vivo and recommend prevention of the formation of oligomers as an attractive therapeutic approach. We have shown that \(\gamma\)-secretase inhibitors can markedly decrease \(A\beta\) oligomer formation by cells at doses that still allow appreciable monomer production [33], and it seems likely that other agents that reduce intracellular monomer levels could have similar effects. Although no physiological function has been confirmed for the \(A\beta\) monomer, substantial or complete depletion of monomers in vivo could potentially result in adverse effects. In contrast, \(A\beta\) oligomers presumably arise solely as a pathological event, hence titrating monomer to levels that cannot support oligomerization or direct targeting of oligomers should relieve the toxic effects of oligomers and also minimize side-effects due to loss of monomer. We have characterized the effects on natural \(A\beta\) oligomerization of a number of compounds known to inhibit \(A\beta\)-mediated toxicity. Thus far we have identified only two compounds capable of inhibiting intracellular oligomer formation and of relieving the oligomer-mediated block of LTP [34]. Both compounds are hydroxyanaline derivatives, which were previously shown to prevent toxicity mediated by synthetic \(A\beta\) [40,41].

The most clinically advanced amyloid-directed therapy, \(A\beta\) immunization, has been shown to reduce cerebral \(A\beta\) levels, decrease amyloid-associated gliosis and neutritic dystrophy, and alleviate memory impairment in transgenic mice [42–46]. Similarly, we have found that the intracerebroventricular injection of anti-\(A\beta\) monoclonal antibodies prevented the oligomer-mediated block of LTP, and that active immunization against \(A\beta\) was partially effective also [47]. Importantly, the degree of protection given by endogenous antibodies was directly related to their ability to recognize \(A\beta\) oligomers. These results suggest that anti-\(A\beta\) antibodies could bind and help clear soluble oligomers of \(A\beta\), so that the latter are no longer present at sufficient concentrations to alter synaptic physiology. Such a mechanism could explain the rapid reversal of cognitive deficits in APP transgenic mice treated acutely with an anti-\(A\beta\) monoclonal antibody [48] and suggests the neutralization of \(A\beta\) oligomers as a potentially powerful mechanism for immunotherapy, distinct from microglial-mediated clearance and peripheral sink effects.

### Conclusions

Adverse effects of \(A\beta\) on hippocampal synaptic plasticity and learned behaviour in vivo can be attributed to a specific,
biochemically defined species of secreted Aβ, namely soluble low-α oligomers. The experimental system described herein should prove useful to further dissect the synaptotoxic effects of Aβ and to test new ways to neutralize them therapeutically. Specifically, agents that reduce Aβ production, or inhibit Aβ aggregation, and antibodies that avidly bind Aβ have already been shown to ameliorate the toxic effects of cell-derived Aβ oligomers, and recommend this paradigm as a useful pre-clinical screen for amyloid-directed therapies.

This work was supported by Wellcome Trust grant 067660 (to D.M.W.), National Institutes of Health (Bethesda, MD, U.S.A.) grant AG05134 (to D.J.S.) and by the Foundation for Neurological Diseases.

References


Received 26 July 2005