Soluble Oligomers of the Amyloid β-Protein: Impair Synaptic Plasticity and Behavior

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Summary. The central quest of research on Alzheimer's disease (AD) is to identify precisely what molecular process first interferes with episodic declarative memory and then prevent that process. During the last 25 years, neuropathological, biochemical, genetic, cell biological and even therapeutic studies in humans have all supported the hypothesis that the gradual cerebral accumulation of soluble and insoluble assemblies of the amyloid β-protein (Aβ) in limbic and association cortices triggers a cascade of biochemical and cellular alterations that produce the clinical phenotype. Missense mutations in presenilin, an unusual intramembrane aspartyl protease, cause rare forms of early-onset AD by altering its cleavage of the amyloid precursor protein (APP) transmembrane domain to increase the ratio of Aβ42 to Aβ40 peptides, thereby promoting Aβ oligomerization. The reasons for elevated cortical Aβ42 levels in most patients with typical, late-onset AD are unknown, but based on recent work, these could turn out to include augmented neuronal release of Aβ during some kinds of synaptic activity. Elevated levels of soluble Aβ42 monomers enable formation of soluble oligomers that can diffuse into synaptic clefts. We have identified certain APP-expressing cultured cell lines that form low-n oligomers intracellularly and release a portion of them into the medium. We find that these naturally secreted soluble oligomers – at picomolar concentrations – can disrupt hippocampal LTP in slices and in vivo and can also impair the memory of a complex learned behavior in rats. Aβ trimers appear to be more potent in disrupting LTP than are dimers. The cell-derived oligomers also decrease dendritic spine density in organotypic hippocampal slice cultures, and this decrease can be prevented by administration of Aβ antibodies or small-molecule modulators of Aβ aggregation. The signaling pathways mediating these effects are under study. Intensive attempts to develop safe and effective Aβ-lowering agents have brought us into human trials that have provided preliminary evidence of cognitive benefit. This therapeutic progress has been accompanied by advances in imaging the Aβ deposits non-invasively in humans. A new diagnostic-therapeutic paradigm to successfully address AD and its harbinger, mild cognitive impairment-amnestic type, is emerging.

Introduction

During most of the 20th century, neurodegenerative diseases remained among the most enigmatic disorders of medicine. The scientific study of these conditions was descriptive in nature, detailing the clinical and neuropathological phenotypes associated with various diseases, but etiologies and pathogenic mechanisms remained obscure. Beginning in the 1970s, advances in two principal areas – biochemical pathology and molecular genetics – combined to yield powerful clues to the molecular underpinnings of several previously "idiopathic" brain disorders. Among the classical neurodegenerative diseases, perhaps the most rapid progress occurred in research on Alzheimer's

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disease (AD). In disorders like Huntington's disease, amyotrophic lateral sclerosis and even Parkinson's disease, unbiased genetic screens, linkage analysis and positional cloning have identified causative genes that subsequently allowed the formulation of specific biochemical hypotheses. In sharp contrast, modern research on AD developed in the opposite order: the identification of the protein subunits of the classical brain lesions guided geneticists to disease-inducing genes, for example, APP, apolipoprotein E and tau. Thus, a biochemical hypothesis of disease – that AD is a progressive cerebral amyloidosis caused by the aggregation of the amyloid β-protein (Aβ) – preceded and enabled the discovery of etiologies.

As progress in deciphering genotype-to-phenotype relationships in AD accelerated during the last two decades, it became apparent that the key challenge for understanding and ultimately treating AD was to focus not on what was killing neurons over the course of the disease but rather on what was interfering subtly and intermittently with episodic declarative memory well before widespread neurodegeneration had occurred (Selkoe 2002). In other words, one wishes to understand the factors underlying early synaptic dysfunction in the hippocampus and then attempt to neutralize these as soon as feasible, perhaps even before a definitive diagnosis of AD can be made. This steady movement of the field toward ever-earlier stages of the disorder is exemplified by the recognition and intensive study of minimal cognitive impairment–amnestic type (MCI; Petersen et al. 1999). And yet patients who die with a diagnosis of MCI have been found to already have a histopathology essentially indistinguishable from classical AD (Price and Morris 1999). Therefore, even earlier phases of this continuum are likely to become recognized, and these might show milder histopathology and might have biochemically, but not yet microscopically, detectable Aβ species that mediate synaptic dysfunction.

The IPSEN symposium for which this volume serves as a record focused on bringing together investigators at the forefront of elucidating the structure and function of hippocampal synapses with investigators focused on understanding how early assemblies of Aβ may compromise some of these synapses. This chapter will summarize some of the observations and discoveries made by the author and his colleagues over several years that have the goal of identifying the earliest synaptotoxic molecules in Alzheimer's disease – and neutralizing them.

Moving from Synthetic Aβ Peptides to Naturally Secreted Aβ Assemblies

A wealth of data from many laboratories now supports the once controversial hypothesis that the accumulation and aggregation of Aβ initiates a complex cascade of molecular and cellular changes that gradually leads to the clinical features of MCI-amnestic type and then frank Alzheimer's disease (Hardy and Selkoe 2002; Hardy and Higgins 1992; Selkoe 1991). As a result, understanding precisely how Aβ accumulation and assembly compromise synaptic structure and function has become the centerpiece of therapeutically oriented research on the disease.

A great many studies have been conducted using synthetic Aβ peptides of either 40 or 42 amino acids, mimicking the two most common lengths of Aβ found in normal human brain and in the cortical and vascular amyloid deposits of AD patients. But

naturally generated Aβ peptides in brain, cerebrospinal fluid (CSF) or the media of cultured cells are considerably more heterogeneous in length (see, e.g., Busciglio et al. 1993; Haass et al. 1992; Iwatsubo et al. 1994; Podlisny et al. 1995; Saido et al. 1995; Seubert et al. 1992; Shoji et al. 1992; Vigo-Pelfrey et al. 1993; Wang et al. 1996). More importantly, almost all studies of synthetic Aβ p peptides have employed concentrations upwards of 1 uM, often 10–40 uM, because the critical concentration allowing relatively rapid assembly of synthetic Aβ40 (the most commonly used peptide) into amyloid fibrils is in the high nanomolar range or greater. Also, synthetic Aβ peptides are often solublized, at least initially, in highly non-physiological solvents (acetonitrile, trifluoroacetic acid, sodium hydroxide, etc.), since these allow the hydrophobic, relatively insoluble peptide to be dissolved and used experimentally.

Many different types of assembly forms of synthetic Aβ, including amyloid fibrils, protofibrils (PF), annular structures, paranuclei, Aβ-derived diffusible ligands and globulomers, have been described over the last two decades (for reviews, see Caughey and Lansbury 2003; Teplow 1998). Even some of the smaller synthetic aggregates do not fulfill the definition of soluble oligomers: Aβ assemblies that are not pelleted from physiological fluids by high-speed centrifugation. For example, protofibrils are intermediates that were observed in the course of studying the fibrillization of synthetic Aβ (Harper et al. 1997; Hartley et al. 1999; Walsh et al. 1999). They are flexible structures that can continue to polymerize in vitro to form amyloid fibrils or can de-polymerize to lower-order species. Protofibrils are narrower than bona-fide amyloid fibrils (∼4–5 nm versus 8–10 nm). Ultrastructural analyses of synthetic protofibril preparat by electron microscopy (EM) and atomic force microscopy (AFM) have revealed both straight and curved assemblies up to 150 nm in length. Synthetic Aβ protofibrils have been shown to contain substantial β-sheet structure, as they are able to bind Congo red or Thioflavin T in ordered fashion.

Annular assemblies of synthetic Aβ are donut-like structures with an outer diameter of 8–12 nm and an inner diameter of 2.0–2.5nm that are distinct from protofibrils by AFM and EM (Bitan and Teplow 2005; Lashuel et al. 2002). Smaller oligomeric species of synthetic Aβ than protofibrils and annuli have been observed, depending on how synthetic Aβ is prepared and incubated, and some have been designated Aβ-derived diffusible ligands (ADDLs; Lambert et al. 1998). Apparent ADDL-like oligomeric assemblies have been isolated from postmortem AD brains and their presence correlated with memory loss (Gong et al. 2003). In separate work, chemical stabilization of synthetic Aβ42 assembly intermediates has revealed an apparent hexamer periodicity, with hexamer, dodecamer, and octadecamer structures observed (Bitan et al. 2003).

In striking contrast to the properties of these various synthetic assemblies, Aβ peptides that are generated in vivo by humans and lower mammals or by cultured cells are diverse with regard to their N- and C-termini, occur naturally in extracellular fluids at low to sub-nanomolar concentrations (Gravina et al. 1995; Näslund et al. 2000; Scheuner et al. 1996; Walsh et al. 2002) and can begin to assemble into metastable dimers, trimers and higher oligomers while still at low nanomolar levels (Podlisny et al. 1995; Walsh et al. 2000). Dimeric, trimeric and apparently tetrameric soluble oligomers have been described in cultured cells (Podlisny et al. 1995; Walsh et al. 2000), and SDS-stable oligomers of varying sizes have also been detected by Western blotting in APP transgenic mouse brain and human brain (Enya et al. 1999; Funato et al. 1999; Kawarabayashi et al. 2004; Lesne et al. 2006; McLean et al. 1999; Roher et al. 1996). Such

natural (i.e., non-synthetic) Aβ oligomers can be resistant not only to SDS but also to chaotropic salts like guanidine hydrochloride and to the Aβ-degrading protease IDE (insulin-degrading enzyme), which can only efficiently digest monomeric Aβ (Walsh et al. 2002). Aβ oligomers produced by cultured cells could be related to the recently described Aβ*56 species, which represents a soluble, SDS-stable dodecamer found in the brains of at least some APP transgenic mouse lines (Lesne et al. 2006). Like the Aβ oligomers produced from cultured cells (Walsh et al. 2002), Aβ∗56 can disrupt synaptic function and thus affect memory (Lesne et al. 2006). Whether Aβ∗56, the cell-derived dimers and trimers (Podlisny et al. 1995) and other soluble oligomers observed in biological systems represent stable assemblies of solely Aβ under native conditions or whether such small oligomeric assemblies stably associate with one or more "carrier" proteins in vivo is currently unclear. Upon further study, Aβ∗56 and Aβ trimers secreted by cultured cells could turn out to share common synaptotoxic properties.

Naturally Secreted Aβ Oligomers Abrogate Hippocampal Synaptic Plasticity

In collaboration with the laboratory of Michael Rowan, we have taken advantage of our discovery that Chinese hamster ovary (CHO) cells stably expressing the AD-causing Val717Phe mutation in APP secrete soluble oligomers detectable on SDS gels as dimers, trimers and tetramers (Podlisny et al. 1995) to conduct a series of studies defining the electrophysiological activities of these assemblies. As mentioned above, we view these cell-derived, low-n oligomers as having several advantages over synthetic Aβ aggregates, most notably their natural production by these and other cells at low- to sub-nanomolar concentrations. Importantly, the CHO-derived oligomers are entirely soluble: centrifuging the conditioned medium at >100 000 g for > hour still leaves all of the monomers and oligomers in the supernatant, with retained neurobiological activity. We have confirmed the identity of these species as bona fide Aβ oligomers using radiosequencing, immunoprecipitation by both N- and C-terminal-specific Aβ monoclonal antibodies, and non-denaturing size exclusion chromatography (SEC). The oligomers can be readily detect in CHO cells stably expressing wild-type human APP (Podlisny et al. 1995), and their disease relevance is supported by our finding that expressing either an APP mutation, a presenilin 1 mutation or a presenilin 2 mutation in CHO cells elevates Aβ42 levels in the media and increases the levels of the SDS-stable dimers and trimers (Xia et al. 1997). We are currently attempting to determine the atomic masses of the oligomers by mass spectrometry to establish their bonding structure; we have already confirmed the mass of the secreted 4 kDa species in the medium as human Aβ monomer, but larger quantities are being prepared to try to obtain the masses of the dimers and trimers.

Dominic Walsh (now at University College Dublin), who led many of our studies characterizing the biochemical and neurobiological properties of the cell-derived oligomers while he was a fellow in my laboratory, established our very productive collaboration with Michael Rowan and his fellow, Igor Kluybin, at Trinity College Dublin. We conducted experiments together to determine whether the monomers and oligomers present in the conditioned medium of the APP V717F-expressing CHO cells

(called 7PA2 cells) can interfere with basal synaptic transmission and/or long-term potentiation (LTP) in the hippocampus in vivo. Through a cannula implanted in the lateral ventricle of adult rats, we administered tiny amounts (usually 1.5–5.0 ul) of the straight conditioned medium collected from the 7PA2 cells. Basal synaptic transmission was unaffected by the medium, but CA1 field recordings after high-frequency stimulation (HFS) of the CA3 Schaeffer collateral-to-CA1 pathway revealed a highly consistent failure to maintain an LTP response in the presence of the 7PA2 medium but not with the medium of untransfected CHO cells (called CHO- cells; Walsh et al. 2002). In the presence of the 7PA2 medium, synaptic potentiation would initially be induced by the HFS but waned over the next 1–3 hours, so that LTP was not maintained. We next performed a number of experiments that indicated that the soluble Aβ monomers in the 7PA2 medium were not responsible for the failure to maintain LTP but the soluble oligomers were. For example, we took advantage of the ability of IDE to degrade the monomers but leave the oligomers (which are surprisingly stable) behind, and this preparation still inhibited hippocampal LTP in vivo. Conversely, injecting the medium after treating the 7PA2 cells with a γ-secretase inhibitor at low doses that reduced the monomers by just ∼40% but thereby decreased dimer/trimer levels by ∼90% led to a normal LTP response. We concluded that secreted, low-n oligomers of human Aβ at picomolar concentrations and in the absence of monomers as well as higher order aggregates (protofibrils, fibrils) could potently inhibit the maintenance of LTP in the mammalian hippocampus.

In recent work, our laboratory has extended our electrophysiological findings to hippocampal slices in vitro. We confirmed the effect of the 7PA2 medium in inhibiting LTP in slices from wild-type mice (Townsend et al. 2006). Further, the cell-derived oligomers blocked a chemically induced form of LTP caused by stimulating dissociated mouse hippocampal neurons with picrotoxin and glycine in the absence of added magnesium (Townsend et al. 2007). The use of the chemically induced LTP paradigm (chem-LTP) on neuronal cultures allowed us to measure the biochemical effects of the soluble oligomers on activation of several different signaling kinases. We found that of five kinases we evaluated, three (ERK MAPK CaMKII and Akt/PKB) had clearly reduced activation upon chem-LTP, whereas two others (PKA and PKC) were unaffected. These data begin to reveal some of the signaling pathways that can be interrupted by naturally secreted oligomers, indicating that there is specificity to the hippocampal response and suggesting that there are discrete receptor-mediated pathways through which soluble oligomers act.

We have also developed protocols for separating the secreted oligomers by SEC under non-denaturing conditions (Klyubin et al. 2005; Townsend et al. 2006; Walsh et al. 2005). When as many as 100 SEC fractions of the 7PA2 conditioned medium were obtained and then tested separately on mouse hippocampal slices, we observed that fractions rich in trimers inhibited LTP even more potently than did fractions enriched principally in dimers; agina, monomers were without effect (Townsend et al. 2006). These results suggest again the existence of a level of molecular specificity in the interactions of Aβ oligomers with neuronal targets. With Dominic Walsh and his colleagues, we are now attempting to purify the oligomers from the CHO cell medium to homogeneity so that they might be labeled and used in ligand binding studies (e.g., by autoradiography) to try to identify the cognate receptors for oligomers but not monomers.

Cell-derived Oligomers Interfere with the Memory of a Complex Learned Behavior

In collaboration with James Cleary and Karen Ashe at the University of Minnesota, we have been able to demonstrate significant cognitive deficits in a complex lever pressing task in adult rats that are directly attributable to a naturally secreted assembly form of Aβ (Cleary et al. 2005). The active Aβ species were the soluble oligomers, not monomers and in the absence of protofibrils and fibrils, and the oligomer effects were characterized by rapid onset, high potency and transience. These combined biochemical and cognitive analyses provided the first direct behavioral data supporting the emerging hypothesis that diffusible oligomers of Aβ are responsible for important components of neuronal dysfunction leading up to or associated with AD (reviewed in Klein et al. 2001; Selkoe 2002; Walsh et al. 2003). The rapid onset and transience of the soluble oligomer effects we saw in the rats are indicative of a pathophysiological action, similar to that seen with amnestic drugs such as scopolamine. Such pathophysiological activities can occur independently of structural neuronal injury and, indeed, our experimental paradigm does not suggest a role for any neurodegenerative processes in the deleterious cognitive effects produced transiently by low picomolar quantities of soluble Aβ oligomers.

In studies reported prior to the Cleary et al. (2005) paper, intracerebral injections of synthetic Aβ that included mixtures of Aβ fibrils, protofibrils, oligomers and monomers in indeterminate proportions exerted deleterious effects on learned behavior in rats (Cleary et al. 1995; Frautschy et al. 2001; O'Hare et al. 1999). However, the deficits were reported to develop over weeks and, once present, they persisted or worsened even without further injections. Because intracerebral injections are usually made during stereotaxic surgery (rather than long after such surgery, as in our paradigm), any immediate acute effects of the injectate on learning or memory typically go unstudied. Two studies attempted to address potential acute effects of very high concentrations of synthetic Aβ species. In one study, increased maze entry errors were observed after i.c.v. injections of 2.0 l of 1 mM synthetic Aβ(1-40) that were given immediately before maze testing (Sweeney et al. 1997). In another study, no effect on intrahippocampal injections of 0.5 µl of 1 mM synthetic Aβ(1-40) that were administered 30 minutes before testing were observed in a radial arm maze test (McDonald et al. 1994). The concentrations and total amounts of synthetic Aβ administered were several orders of magnitude higher than those of the natural, cell-secreted oligomers used in our study (Cleary et al. 2005). Also, the duration between administration and testing was different. Because the assembly sizes of the synthetic Aβ that was injected were not defined in either study, the specific forms of Aβ being tested could not be deduced, in contrast to the present work.

Our work may provide an explanation for the observation that subtle brain dysfunction can be detected in certain individuals who are genetically at risk for AD but remain neurologically stable for many years before the expected onset of disease (Bookheimer et al. 2000; Small et al. 2000). In addition, our linkage of acute behavioral deficits with soluble oligomers provides a mechanistic explanation for the rapid restoration of cognitive function that followed administration of anti-Aβ antibodies in behaviorally impaired APP transgenic mice (Dodart et al. 2002; Kotilinek et al. 2002).

Cell-Derived Oligomers Decrease Dendritic Spine Density in Hippocampus by an NMDA-Dependent Signaling Pathway

Our next approach to deciphering the synaptic effects of natural Aβ oligomers was to ask whether they can induce structural alterations of synapses in association with the clear functional deficits described in the previous two sections above. We exposed organotypic rat hippocampal slice cultures that had been biolistically transfected with EGFP to subnanomolar concentrations of SEC-separated 7PA2 cell Aβ monomers or dimers/trimers for periods varying from 1 to 15 days. We observed a marked decrease in dendritic spine density in EGFP-positive pyramidal neurons that reached ∼60% loss after 15 days of exposure (Shankar et al. 2007). Electrophysiological experiments confirmed that the decreased spine density reflected a loss of excitatory synapses. Miniature EPSCs were measured from neurons in hippocampal slices treated for 10–15 days. mEPSC amplitude, which reflects the postsynaptic AMPA-type glutamate receptor (AMPAR)-mediated response to the release of a single vesicle of glutamate, was slightly but significantly reduced in oligomer-treated neurons compared to control-medium treated neurons. Moreover, as expected from the loss of dendritic spines, the inter-mEPSC interval was significantly increased (195 ± 5 ms vs. 85 ± 3 ms), corresponding to a ∼50% reduction in mEPSC frequency (Shankar et al. 2007).

The strong decrease in dendritic spine density was reversible when the Aβ oligomer-rich medium (which had been applied for 10 days) was exchanged for normal slice medium for 5 days; spine density returned to almost normal levels (Shankar et al. 2007). Spine loss was also fully prevented by co-application to the slices of an N-terminal monoclonal antibody to Aβ (6E10) together with the soluble oligomers; denatured (boiled) 6E10 had no rescue effect. We also examined s*cyllo*-inositol (AZD-103), a *myo*-inositol steroisomer shown by Joanne McLaurin and colleagues to decrease Aβ plaque number and size and prevent behavioral deficits in an APP transgenic mouse line (McLaurin et al. 2006). At 5 uM concentrations, *scyllo*-inositol co-administered with the soluble oligomers fully prevented the spine loss, whereas a known inactive stereoisomer, *chiro*-inositol, did nothing.

Acute application of synthetic Aβ can trigger a rapid reduction of NMDAR-dependent currents in dissociated cortical neurons that is thought to result from Aβ-mediated activation of nicotinic acetylcholine receptors (nAchRs; Snyder et al. 2005). To determine if the same signaling pathway was responsible for the Aβ oligomer-mediated spine loss, we examined the ability of NMDAR and nAchR antagonists to mimic or block the effects of secreted Aβ. Chronic blockade of nAchRs by 10-day exposure to the irreversible antagonist α-Bungarotoxin had no effect on the spine density of control neurons and did not affect the degree of spine loss produced by oligomer application. Conversely, although 10 days of application of the NMDAR antagonist CPP (20 μM) alone had no effect on spine density, it completely prevented the spine loss normally seen with Aβ oligomer incubation, indicating that NMDAR-activity is required for Aβ-mediated spine loss (Shankar et al. 2007).

To determine if Aβ oligomers reduce NMDAR-mediated synaptic Ca influx in hippocampal pyramidal neurons, 2-photon laser photoactivation (2PLP) of caged (MNI)-glutamate was used to stimulate the postsynaptic terminal on a visualized spine while evoked calcium transients were measured in the spine head with 2P laser scanning microscopy (Bloodgood and Sabatini 2007). Whole-cell recordings were obtained from

CA1 pyramidal neurons in acute hippocampal slices, and cells were filled through the patch pipette with the Ca-sensitive, green-fluorescing fluorophore Fluo-5F and the Ca-independent, red-fluorescing fluorophore Alexa Fluor-594. Red fluorescence was used to visualize morphology and to select spines within the proximal 150 µm of an apical dendrite for analysis. Uncaging-evoked fluorescence transients were monitored in the spine head and adjacent dendrite while uncaging-evoked postsynaptic potentials (uEPSPs) were recorded at the soma. Fluorescence transients were quantified relative to maximal green fluorescence at saturating levels of Ca ($\Delta G_{uEPSP}/G_{sat}$), a measure that, under our recording conditions, is linearly proportional to evoked changes in Ca ($\Delta[Ca]_{uEPSP}$; Bloodgood and Sabatini 2007). We found that $\Delta[Ca]_{uEPSP}$ was smaller in the presence of Aβ oligomers ($\Delta G_{uEPSP}/G_{sat} = 7.4 \pm 0.5\%$, 21/5 spines/cells) than in control conditions ($\Delta G_{uEPSP}/G_{sat} = 10.2 \pm 1.1\%$, 20/7 spines/cells, $p < 0.05$). In contrast, $\Delta[Ca]_{uEPSP}$ in the presence of Aβ monomers ($\Delta G_{uEPSP}/G_{sat} = 9.9 \pm 1.0\%$, 19 spines/6 cells) was the same as in control conditions. Thus, exposure to picomolar levels of soluble Aβ oligomers acutely reduces but does not abolish $\Delta[Ca]_{uEPSP}$, consistent with a partial reduction of NMDAR-mediated Ca influx into active spines.

Partial blockade of NMDARs and reduced Ca^{2+} influx through NMDARs favor the induction of long-term depression (LTD) via a calcineurin-dependent pathway (Cummings et al. 1996; Mulkey et al. 1994)). LTD induction is accompanied by a shrinkage of dendritic spines that is mediated by regulation of the actin-depolymerization factor cofilin (Shankar et al. 2007; Zhou et al. 2004) at a conserved serine (position 3) that can be phosphorylated by LIM-kinase. We found that neurons transfected with a plasmid encoding cofilin S3D, in which serine 3 is replaced by the phosphomimetic amino acid aspartate, rendering cofilin constitutively inactive, displayed a net increase in dendritic spine density. Expression of this construct also prevented the loss of dendritic spines normally seen following a 10-day exposure to Aβ oligomers. To examine whether Aβ oligomers mediate spine loss through activation of calcineurin, we examined the ability of FK506 to block Aβ oligomer-induced spine loss. Incubation in FK506 (1 uM) alone for 24 hr had no effect on spine density. However, co-administration of FK506 with Aβ oligomers fully prevented spine loss (Shankar et al. 2007).

Conclusions

Through a series of systematic studies of soluble oligomers of human Aβ secreted by cultured cells, we have documented that low-n oligomers – but not monomers from the same source and at higher concentrations – can inhibit LTP without affecting basal synaptic transmission, can reversibly alter the structure of excitatory synapses by decreasing spines, and can interfere with the memory of a learned behavior in healthy adult rats. We interpret these data to signify that small diffusible oligomers impair both synaptic function and synaptic structure on glutamatergic neurons in the hippocampus. Therapeutically, we show that oligomer-mediated spine loss is prevented by antibodies to Aβ and a small molecule inhibitor of Aβ aggregation. We had previously reported that both active and passive immunotherapy of normal adult rats protect these animals form the LTP-inhibiting effects of soluble Aβ oligomers administered intracerebroventricularly (Klyubin et al. 2005). Mechanistically, we find that dendritic spine reduction requires activity of a signaling cascade involving NMDARs, calcineurin,

and cofilin. These results suggest that Aβ oligomers shift the activation of NMDAR-dependent signaling cascades towards pathways involved in the induction of LTD. We propose that chronic activation of these pathways by soluble Aβ oligomers found in the brains of patients with AD contributes to the pathogenesis of the disease and may underlie the synapse loss in hippocampus that occurs early in the disease process and is a key quantitative correlate of degree of cognitive impairment in AD patients (Davies et al. 1987; Masliah et al. 2001; Terry et al. 1991).

Overall, our studies with soluble natural oligomers suggest a model (Fig. 1) in which exposure to Aβ oligomers mimics a state of partial NMDAR blockade, either by reducing NMDAR activation, reducing NMDAR-dependent Ca influx, or enhancing NMDAR-dependent activation of calcineurin. We propose that these effects promote the LTD-inducing mode and inhibit the LTP-inducing mode of NMDAR-dependent signaling. Since LTP induction promotes spine enlargement and the growth of new spines whereas LTD induction promotes spine shrinkage and retraction (Engert and Bonhoeffer 1999; Maletic-Savatic et al. 1999; Matsuzaki et al. 2004; Nagerl et al. 2004; Zhou et al. 2004), this oligomer-induced imbalance would promote the progressive loss of dendritic spines and glutamatergic synapses. Furthermore, a previous study reported that FK506 prevented the ability of synthetic Aβ to inhibit LTP in hippocampus (Chen et al. 2002), suggesting that the inhibition of LTP by Aβ oligomers reflects a shift in the LTP/LTD balance rather than a direct blockade of LTP-inducing pathways.

Our proposed model is largely consistent with a recent report that also concludes that Aβ decreases dendritic spine density and alters glutamatergic signaling in CA1 pyramidal neurons in rat hippocampal organotypic slices (Hsieh et al. 2006). Hsieh et al. found that neurons overexpressing human APP have decreased dendritic spine density compared to control neurons. Furthermore, transduction with a C-terminal fragment of APP (β-CTF) decreased AMPAR-mediated synaptic currents. This effect mimicked and partially occluded metabotropic glutamate receptor-induced LTD, involving endocytosis of GluR2-containing AMPARs. Important differences between the two studies may explain why we found no decrease in AMPAR-mediated mEPSCs whereas Hsieh et al. found a ~30% reduction in AMPAR EPSCs. For example, Hsieh et al. performed recordings ~24 hours after transduction with β-CTF and therefore described effects of short-term exposure to Aβ that may be mechanistically different from the longer-term effects described here. Furthermore, Hsieh et al. found alterations in synaptic transmission in neurons expressing β-CTF but not in neighboring cells, suggesting cell-autonomous or autocrine effects of APP or Aβ. Aβ in their system is generated intracellularly and secreted, so at least some of the observed effects could be attributable to Aβ acting intracellularly, whereas our system involves extracellular application of secreted Aβ. The APP transduction paradigm used by Hsieh et al. does not allow a distinction between the effects of Aβ oligomers and monomers, whereas we are able to ascribe the synaptic changes specifically to soluble oligomers of Aβ. However, despite the differences in the design and analysis performed in the two studies, both support a model in which Aβ perturbs excitatory synapses by enhancing LTD in an activity- and calcineurin-dependent manner.

The next step in this line of research is to translate this approach and the resultant findings to the human disease. We must determine whether soluble Aβ monomers and oligomers isolated directly from the cerebral cortex of typical, late-onset AD cases can induce the same structural and functional deficits and whether they do so by
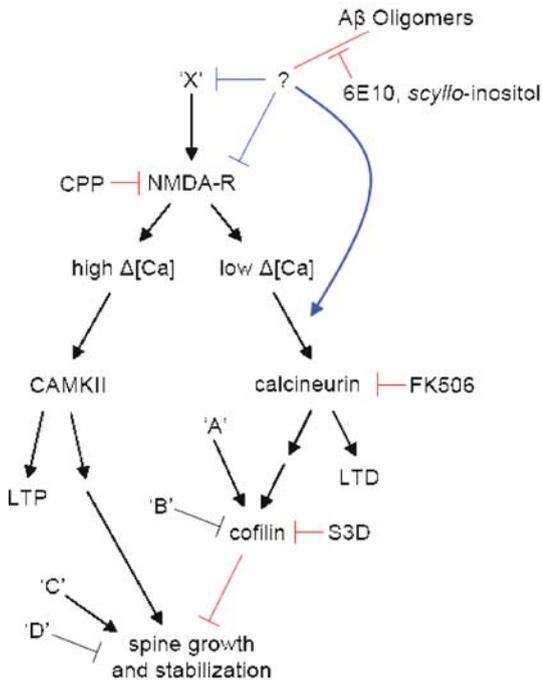
Fig. 1. Proposed pathways that regulate spine density and that are affected by Aβ oligomers, based on the results of our results. Ca^{2+} influx through synaptic NMDARs can activate at least two pathways that regulate spine density. On the left-hand side, high levels of Ca^{2+} accumulation, such as those reached during tetanic or suprathreshold synaptic stimulation, induce LTP via a CAMKII-dependent pathway (reviewed in Nicoll and Malenka 1999). LTP-inducing stimuli also trigger the enlargement of dendritic spines and growth of new spines in a NMDAR- and CAMKII-dependent manner (Engert and Bonhoeffer 1999; Jourdain et al. 2003; Maletic-Savatic et al. 1999; Matsuzaki et al. 2004; Nagerl et al. 2004). Introduction of active CAMKII in neurons is sufficient to induce new spine growth (Jourdain et al. 2003). In the right-hand side pathway, low levels of Ca^{2+} accumulation, such as those reached during low-frequency subthreshold stimulation, induce LTD through a calcineurin-dependent pathway (reviewed in Malenka and Bear 2004). LTD-inducing stimuli also lead to spine shrinkage via an NMDAR/calcineurin/cofilin-dependent pathway and spine retraction through an NMDAR-dependent pathway (Nagerl et al. 2004; Zhou et al. 2004). The calcineurin and cofilin dependence of LTD-associated spine retraction have not been examined. In this model, full block of NMDARs interrupts both pathways, leading to no net spine loss. Partial block of NMDARs favors activation of the right-hand pathway, LTD induction, and loss of spines. In addition, multiple factors (A, B, C, and D) act independently of NMDARs, CAMKII, and calcineurin to regulate cofilin and spine density. We find that soluble Aβ oligomers decrease spine density in an NMDAR/calcineurin/cofilin-dependent manner, consistent with activation of the pathway shown on the right. Aβ oligomers reduce NMDAR-dependent Ca^{2+} transients, possibly shifting stimuli that normally activate the left-hand pathway to instead activate those on the right. This activation might occur through direct interaction of Aβ with NMDARs or by first activating unknown factors (X) that may lead to inhibition of NMDAR-mediated synaptic Ca^{2+} influx. Aβ may also facilitate NMDAR-dependent activation of calcineurin via additional pathways. *Blue lines* indicate levels at which soluble Aβ oligomers may modulate the pathway and *red lines* indicate elements of the pathway tested in this study

similar signaling mechanisms as summarized above. If this can be achieved, we may be able to conclude that we have identified the "smoking gun" that induces synaptic impairment in the brains of Alzheimer's patients. In a sense, this next step would be attempting to fulfil one of Koch's postulates for identifying the causative agent of an idiopathic disease: isolating the putative agent, transmitting it to normal brain tissue (of a rodent, as this cannot be done in humans), and showing that the agent reproduces the key phenotypic features of the disorder. If such experiments are successful, they will strongly validate and encourage the current intensive effort to treat, and perhaps ultimately prevent, AD using agents that lower Aβ in the brain.

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