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Review

Amyloid flirting with synaptic failure: Towards a comprehensive view of Alzheimer's disease pathogenesis

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Abstract

Many neurological disorders accompanied by cognitive deficits exhibit abnormal synaptic function. This emerging concept is exemplified by Alzheimer's disease. According to the amyloid hypothesis, Alzheimer's disease is thought to be caused by the progressive accumulation and deposition of neurotoxic Amyloid β-peptide in amyloid plaques and aggregates in brain. Now new theories are emerging associating synaptic and neuronal loss to Amyloid β monomers and Amyloid β oligomers. In particular, Amyloid β oligomers have been described as the earliest effectors to adversely affect synaptic structure and plasticity. In this way, they compromise aspects of learning and memory, including long-term potentiation. Local inflammatory changes, neurofibrillary degeneration, and neurotransmitter deficits all contribute to the memory impairment, but available evidence suggests that these alterations develop as a consequence of early Amyloid β accumulation. Even more recently, different studies have focused on the capability of neuronal activity itself to influence Amyloid Precursor Protein (APP) metabolism. Neuronal activity modulates, in fact, the formation and secretion of Amyloid β peptides. The identification of both the mechanism through which Amyloid β can modify neuronal activity and the way by which neuronal activity can alter APP metabolism is becoming more and more important. And the challenge for the future is, therefore, to find the linkage between these two processes.

Keywords: Alzheimer's disease; Synaptic function; Amyloid β

Contents

1. Introduction

The dramatic rise in life expectancy during the 20th century has resulted in a growing number of individuals achieving the age at which neurodegenerative disorders become common.

Among these, Alzheimer's disease has emerged as the prevalent form of late-life mental failure in humans.

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Alzheimer's disease is a progressive dementia that manifests in early stages as a profound inability to form new memories (Selkoe, 2002).

The syndrome is the result of abnormalities associated with dysfunction and death of specific population of neurons, particularly those cells in neural systems participating in memory and cognitive functions (Price et al., 1998).

The neuropathology is characterized by the presence of intracellular and extracellular protein or peptide aggregates: the hyperphosphorylated tau, assembled into the paired helical filaments within neurofibrillary tangles and swollen neuritis (Grundke-Iqbal et al., 1986), and the Amyloid β-peptides existing in extracellular β-pleated sheet conformations assembled into oligomers, in amyloid plaques (Glenner and Wong, 1984).

For many years, this pathology was attributed to nerve-cell death induced by deposits of fibrillar Amyloid β (Hardy and Higgins, 1992). The cloning of the gene encoding the Amyloid Precursor Protein (APP) allowed to localize it on chromosome 21 (Kang et al., 1987; Goldgaber et al., 1987; Tanzi et al., 1987; Robakis et al., 1987). This information coupled with the earlier recognition that trisomy 21 (Downs syndrome) leads invariably to the neuropathology of Alzheimer's disease (Olson and Shaw, 1969), set the stage for the proposal that Amyloid β accumulation is the primary event in Alzheimer's disease pathogenesis.

A new understanding of the amyloid cascade hypothesis proposes an alternative mechanism for memory loss based on the impact of small, soluble Amyloid β oligomers (Hardy and Selkoe, 2002; Klein et al., 2001). This hypothesis emerged from experiments showing that Amyloid β oligomers rapidly inhibit long-term potentiation, a classic experimental paradigm for memory and synaptic plasticity (Lambert et al., 1998; Walsh et al., 2002; Wang et al., 2002).

Indeed, early memory loss originates from synapse failure before neuron death and synapse failure derives from actions of Amyloid β oligomers rather than fibrils. In support of this hypothesis, many studies have demonstrated that the best statistical correlation occurs between measures of synaptic density and degree of dementia (DeKosky and Scheff, 1990; Terry et al., 1991), and have documented a significant decrease in synaptic density in the association cortices and hippocampus of Alzheimer's disease brain (Bertoni-Freddari et al., 1996; Davies et al., 1987; DeKosky and Scheff, 1990; Masliah et al., 2001; Terry et al., 1991). Moreover, the decrease in synapse number and density seems disproportionate to the loss of neuronal cell bodies (Davies et al., 1987; DeKosky and Scheff, 1990; Bertoni-Freddari et al., 1996), suggesting that pruning of synaptic endings may precede the demise of the neuron in the disease process. Furthermore, some changes in the brains of Alzheimer's disease patients and APP transgenic mice suggest that synaptic function is compromised prior to the physical degeneration of the synapses (Palop et al., 2003; Westphalen et al., 2003; Yao et al., 2003).

In this scenario, this review will portray the state of the art of the correlation existing between Amyloid β and synaptic function.

2. A snapshot of the main character of the pathogenesis: the amyloid cascade

Molecular pathogenesis of neurodegenerative diseases is the result of a complex interplay of several crossing pathways, involving primary and secondary events (Bossy-Wetzel et al., 2004). In the case of Alzheimer's disease, accumulating evidence indicates the amyloid cascade as the primary event in the pathogenesis (Selkoe, 2000).Therefore, the mechanisms leading to Amyloid β production were investigated in minute details to determine the etiology of the disease.

In the late 1980s, it was first recognized that the Amyloid β peptide is derived from its large precursor protein, APP, by sequential proteolytic cleavages (Kang et al., 1987).

Amyloid β production turned out to be one paradigmatic example of a more general biological process called regulated intramembrane proteolysis. Membrane proteins, as APP or Notch, firstly undergo a shedding process leading to the release of ectodomains in extracellular fluids. Secondly, the membrane-retained stubs can be cleaved within their intracellular domains giving rise to small hydrophobic peptides released into extracellular fluids as well as to intracellular domains into the cytoplasm. These small intracytoplasmic peptides may possess different functions including activation of nuclear signaling (Haass, 2004).

In the case of APP the shedding process is mediated by α- or β- secretase, while the cleavage of the membrane-retained stubs is due to γ-secretase (Haass and Selkoe, 1993).

β-Secretase activity generates the NH_2-terminus of Amyloid β (Seubert et al., 1993), cleaving APP to produce a soluble version of APP (sAPPβ) and a 99-residue COOH-terminal fragment (CTF99 or β-stub) that remains membrane bound. In contrast, α-secretase cuts within the Amyloid β region (between residues Lys16 and Leu17 of Amyloid β) to produce a soluble fragment, (sAPPα) and an 83-residue COOH-terminal fragment (CTF83 or α-stub) (Esch et al., 1990; Sisodia, 1992). Both CTF99 and CTF83 are substrates of γ-secretase. The γ-secretase complex performs an unusual proteolysis in the middle of their transmembrane domains to produce the 4-kDa Amyloid β from CTF99 and a 3-kDa peptide, called p3, from CTF83 (Haass et al., 1992; Haass and Selkoe, 1993) as well as the APP intracellular domain which can activate nuclear signaling. Proteolysis by γ-secretase is heterogeneous: most of the full-length Amyloid β species produced is a 40-residues peptide (Amyloid β_{40}), whereas a small proportion is a 42-residues COOH-terminal variant (Amyloid β_{42}) (Esler and Wolfe, 2001) (Fig. 1).

All Alzheimer's disease-causing APP mutations which have been identified occur either within or flanking the Amyloid β region. In particular, mutations which flank Amyloid β domain increase the production of the highly toxic Amyloid β_{42} (Haass, 2004). The generation of Amyloid β species with variable hydrophobic C-terminus leads to different properties of oligomerization and self-aggregation and, therefore, it is directly relevant for Alzheimer's disease onset and progression (Haass, 2004).

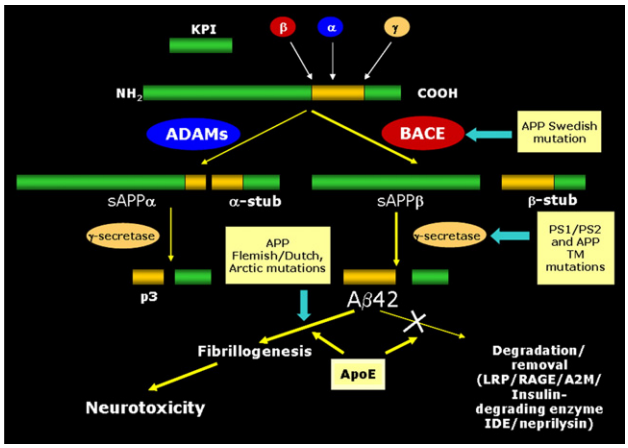

Fig. 1. APP metabolism and Amyloid β formation.

The players of the amyloid cascade have been identified in the past two decades.

β-site APP-cleaving enzyme 1 (BACE1) is clearly the only protease with a well-defined β-secretase activity. This was unambiguously shown by the homozygous knock out of BACE1 gene, which does not allow any Amyloid β generation (Cai et al., 2001; Luo et al., 2003).

α-secretase is the main protagonist of the physiological APP metabolic pathway. A disintegrin and metalloproteinase 10 (ADAM10), the most accredited candidate for α-secretase (Postina et al., 2004), was first isolated from bovine brain as an enzyme capable of degrading myelin basic protein; subsequent isolation and sequencing of its cDNA revealed that it is a member of the A Disintegrin And Metalloproteinase (ADAMs) family and the enzyme was referred to as ADAM10 (Lammich et al., 1999).

To evaluate the involvement of these two enzymes in APP metabolism, several recent studies have examined the expression of ADAM10 and BACE1 in Alzheimer's disease patients. As expected, ADAM10 protein levels were found to be significantly reduced in platelets of sporadic Alzheimer's disease patients, and both BACE1 and ADAM10 activity were shown respectively increased and reduced in platelets as well as in temporal cortex homogenates obtained from Alzheimer's disease patients compared to control subjects (Colciaghi et al., 2004).

γ-Secretase is required for the rather unusual intramembrane cleavage of CTF99 and CTF83, a process initially thought to be biochemically impossible. A set of four proteins is essential to build up the γ-secretase complex (De Strooper, 2003). The two homologous presenilins (PSs), Presenilin 1 (PS1) and Presenilin 2 (PS2), are genetically linked to numerous cases of familial Alzheimer's disease (Sherrington et al., 1995) and are apparently of exceptional importance for the γ-secretase cleavage event. More than 100 autosomal dominant PSs point mutations have been now identified, and are all able to cause aggressive early-onset Alzheimer's disease by the same mechanism.

PSs alone are not sufficient for γ-secretase activity, and require additional cofactors to form a multiprotein high-molecular-weight active complex (De Strooper, 2003). Biochemical purification of this complex led to the identification of nicastrin (Nct) (Yu et al., 2000). The remaining components of the complex were isolated by genetic screenings for enhancers of a PS-dependent Notch-deficient phenotype in *Caenorhabditis elegans*: anterior pharynx-defective phenotype (APH-1) and PS-enhancer (PEN-2) (Francis et al., 2002; Goutte, 2002; De Strooper, 2003).

When all four components are expressed together in *Saccharomyces cerevisiae*, an organism that lacks any endogenous γ-secretase activity, fully active γ-secretase is reconstituted (Edbauer et al., 2003).

3. APP metabolites: sAPPα and synaptic plasticity

The functions of APP and its homologues *in vivo* remain poorly understood. The overall structure of the protein suggests that APP could be a receptor or a growth factor (Rossjohn et al., 1999). In neuronal cells full-length APP may play important roles in nerve cell structure and signal transduction.

In the case of APP-null mutations, mice show a variety of alterations in neural structure and function, including gliosis, decreased neocortical and hippocampal levels of synaptophysin, lowered dendritic lengths in hippocampal neurons, reduced survival of cultured neurons and impaired long-term potentiation (Perez et al., 1997; Dawson et al., 1999; Seabrook and Rosahl, 1999). However, these effects could be due as much to the loss of APP neurotrophic derivative sAPPα, as to the loss of activity by full-length APP. It is important to know, therefore, whether administration of sAPPα can rescue these deficits in APP-deficient mice.

Indeed, secreted APP exerts proliferative actions in a variety of cell types as well as neurotrophic and neuroprotective effects (Mucke et al., 1996). In general, these effects are induced by sAPPα approximately 100 times more strongly than by sAPPβ. In one study examining neurite outgrowth, sAPPβ actually lowered growth below control levels (Li et al., 1997).

The way by which sAPPα exerts its effects is still unknown. However, sAPPα has many putative binding partners (serum proteins, cell surface proteins and extracellular matrix proteins) and could affect neuronal physiology and structure. At low concentrations (1 nM), exogenous sAPPα potently inhibits the excitability of cultured hippocampal neurons by hyperpolarizing the membrane and activating at least one potassium current (Furukawa et al., 1996a).

In addition to influencing membrane excitability, sAPPα also regulates calcium homeostasis by reducing resting free calcium levels in cultured hippocampal neuron soma (Mattson et al., 1993). This effect may be a consequence of activating potassium channels, since potassium channel agonists produced similar decreases and under these conditions sAPPα no longer lowered resting Ca^{2+} (Furukawa et al., 1996b).

A separate result of sAPPα application to cultured neurons is the down-regulation of *N*-methyl D-aspartate (NMDA) receptor-mediated currents (Furukawa and Mattson, 1998). The NMDA receptor regulation was inhibited by a C-terminal

antibody against sAPPα, by protein phosphatase inhibitors and by Protein Kinase G inhibitors. The effect of sAPPα was specific to NMDA receptors, as α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptor currents were not affected. Whether sAPPα influences receptor function of other neurotransmitter systems remains to be investigated (Furukawa and Mattson, 1998). This effect on NMDA receptor shows sAPPα neuroprotective action and is consistent with other studies in which NMDA receptor antagonist turned out to be effective in slowing cognitive decline in mild to severe Alzheimer's disease patients (Wilcock, 2003).

One study examined the role of the sAPPα fragment on synaptic plasticity. The authors observed that incubation of rat hippocampal slices for 1 h with 100 nM sAPPα produced a complex pattern of effects on long-term potentiation and long-term depression in the hippocampal area CA1. In control slices, 1 Hz Low Frequency Stimulation induced long-term depression, 10 Hz stimulation produced no net change in synaptic efficacy while 100 Hz High Frequency Stimulation produced long-term potentiation.

In sAPPα-treated slices, long-term depression was blocked at 1 Hz but facilitated at 10 Hz. Long-term potentiation induced by 100 Hz stimulation was also facilitated. The effect of sAPPα thus appeared to be one of raising the threshold for long-term depression but facilitating long-term potentiation (Ishida et al., 1997). This pattern of changes bears some similarity to that seen in synapses that have previously been depressed (Ngezahayo et al., 2000).

Secreted forms of APP_{751} and APP_{695} have potent memory-enhancing effects and block learning deficits induced by scopolamine: in behavioural paradigms, intracerebroventricularly administered sAPPα enhanced memory in normal and amnesic mice (Meziane et al., 1998). Furthermore, a positive association between cerebrospinal fluid levels of sAPPα and cognitive performance in rats has been reported (Anderson et al., 1999).

In a recent study, Postina and collaborators demonstrate that ADAM10 overexpression in an Alzheimer's disease animal model reverses impaired long-term potentiation and cognitive deficits early in life before plaque formation occurs. As reported (Dewachter et al., 2002), a neuron-specific knockout of PS1 prevented amyloid plaque formation, but did not improve cognitive deficits of APP[V717I] mice, the mouse model used in the Postina investigation. In their study, the beneficial effect of increased ADAM10 activity, including cognitive improvements, can most likely be attributed to the combined effects of decreased levels of toxic Amyloid β peptides and endogenously increased amounts of neuroprotective sAPPα (Postina et al., 2004).

In contrast, another study shows that the limited overproduction of APP cleavage products, at levels that do not lead to the formation of Amyloid β plaques or oligomers, can improve the retention of spatial memory and enhance activity-dependent forms of long-lasting and short-term synaptic plasticity (Ma et al., 2007). The enhanced memory and synaptic plasticity depend on BACE1-mediated APP processing and correlate with elevated levels of APP intracellular domain, but not with other APP cleavage products. These data suggest that the regulated, activity-dependent cleavage of APP by BACE1 in neurons may facilitate aspects of normal brain function, in sharp contrast with previous studies in culture, which showed that synaptic function is inhibited by BACE1-mediated cleavage of APP (Kamenetz et al., 2003).

Moreover, they dissociated the enhanced memory and synaptic plasticity in Transgenic APP (TgAPP) mice from sAPPα, which has been reported to enhance memory and synaptic plasticity in rats (Ishida et al., 1997; Meziane et al., 1998). Their findings are consistent with recent studies showing that sAPPα infused into the lateral ventricles of rats does not enhance long-term potentiation or cognitive function (Walsh et al., 2002; Cleary et al., 2005). However, these negative results do not exclude other potential positive effects of sAPPα on brain function, which may be elicited only under specific testing conditions.

4. The other side of the coin: Amyloid β and synaptic failure

Diverse lines of evidence now suggest that Amyloid β plays a central role in the pathogenesis of neuronal dysfunction in Alzheimer's disease (Selkoe, 1991; Hardy and Selkoe, 2002). In Alzheimer's disease patients, memory impairment strongly correlates with cortical levels of soluble Amyloid β species, including oligomers (Lue et al., 1999; McLean et al., 1999; Wang et al., 1999).

A pivotal question is how Amyloid β could affect synaptic activity.

Several electrophysiological studies of young mice transgenic for human APP with Alzheimer's disease-causing mutations have revealed significant deficits in basal synaptic transmission and/or long-term potentiation in the hippocampus, well before the development of microscopically detectable Amyloid β deposits (Larson et al., 1999; Moechars et al., 1999).

For example, in a study of a line bearing the Lys^{670}→Asn, Met^{671}→Leu double mutation in APP sequence, no changes in basal synaptic transmission were observed at age 2 to 8 months (no Amyloid β deposits) or at age 15 to 17 months (many Amyloid β deposits), but hippocampal long-term potentiation measured *in vivo* became severely impaired at the latter age (Chapman et al., 1999). The long-term potentiation deficit in these older mice was associated with impaired performance in a spatial working memory task but little or no loss of certain synaptic markers, suggesting that functional and not structural-synaptic changes were responsible for the cognitive deficits. A separate study in hippocampal slices taken from the same line found decreased basal synaptic transmission but no change in long-term potentiation at ages 12 and 18 months (Fitzjohn et al., 2001).

However, Amyloid β neurotoxicity can be mediated by multiple different assembly forms of the peptide itself. The nature of the synaptotoxic Amyloid β species in the brain is very difficult to define because the animals accumulate a mixture of Amyloid β forms (monomers, soluble oligomers, insoluble oligomers, and some insoluble amyloid fibrils) that are likely to exist in dynamic equilibrium.

Although Amyloid β is the subunit of the amyloid progressively deposited in myriad neuritic plaques in the limbic and association cortices of all Alzheimer's disease patients, it might play a role in memory impairment also in a soluble or oligomeric state. Therefore, studies on Amyloid β toxicity cannot ignore the aggregation state of the detected species.

This evidence comes from analyses of human brains that demonstrated robust correlations between cortical levels of soluble Amyloid β and the extent of synaptic loss and severity of cognitive impairment (Lue et al., 1999; McLean et al., 1999; Wang et al., 1999). In one study, when a buffer-soluble fraction of Alzheimer's disease cerebral cortex was examined by sensitive Western blotting, not only monomeric Amyloid β (4 kDa) but also sodium dodecyl sulfate stable oligomers (8 and 12 kDa) were detected (McLean et al., 1999). Similar oligomers have also been detected in the hippocampal CA1 region and entorhinal cortex of humans in the absence of amyloid plaques and, in many cases, in the absence of neurofibrillary tangles. The latter result suggests that the accumulation of Amyloid β oligomers may occur very early in the disease process (Funato et al., 1999).

Therefore, Amyloid β assembles into a variety of higher-order structures that include dimers, oligomers, protofibrils and fibrils, and evidence has emerged to support a role for soluble oligomeric and protofibrillar forms in dysregulation of synaptic function.

Several studies aimed to identify which one of these different assemblies is responsible for synaptic dysfunction and memory deficit.

Experiments performed by Selkoe and coworkers allow inhibition of hippocampal long-term potentiation *in vivo* to be attributed specifically to soluble oligomers, not monomers or fibrils, of secreted human Amyloid β (Walsh et al., 2002). In certain cultured cell lines expressing mutant human APP, natural oligomers of human Amyloid β are formed soon after generation of the peptide within intracellular vesicles and are later secreted from the cell at low nanomolar levels. Intracerebroventricular microinjection of cell medium containing these oligomers and abundant monomers (but no amyloid fibrils) potently inhibited hippocampal long-term potentiation in adult rats. This inhibition occurs at low to sub-nanomolar concentrations that are similar to those found in human cerebrospinal fluid. Immunodepletion from the medium of all Amyloid β species abrogated the long-term potentiation block. Pretreatment of the medium with a protease that selectively degrades Amyloid β monomers but not oligomers failed to prevent the long-term potentiation inhibition (Walsh et al., 2002).

In particular, a detailed electrophysiological characterization of the effects of secreted human Amyloid β, separated by size-exclusion chromatography, on hippocampal synaptic plasticity shows that a trimer specie is a particularly potent inhibitor of long-term potentiation (Townsend et al., 2006).

Moreover, in a recent study, Tg2576 mice, which express an APP variant linked to Alzheimer's disease, were used to investigate the cause of memory decline in the absence of neurodegeneration or Amyloid β protein amyloidosis. Young Tg2576 mice (<6 months old) have normal memory and lack neuropathology, middle-aged mice (6–14 months old) develop memory deficits without neuronal loss, and old mice (>14 months old) form abundant neuritic plaques containing amyloid-β (Hsiao et al., 1996; Irizarry et al., 1997; Kawarabayashi et al., 2001; Westerman et al., 2002).

Karen Ashe and coworkers combined behavioural studies with a detailed biochemical analysis of the Amyloid β assemblies found in the brains of Tg2576 mice.

Memory deficits in middle-aged Tg2576 mice turned out to be caused by the extracellular accumulation of a 56-kDa soluble Amyloid β assembly, which was termed Amyloid β∗56.

Amyloid β∗56 purified from the brains of impaired Tg2576 mice disrupts memory when administered to young rats. They proposed that Amyloid β∗56 impairs memory independently of plaques or neuronal loss, and may contribute to cognitive deficits associated with Alzheimer's disease (Lesne et al., 2006).

Therefore, the identification of a single cytopathological Amyloid β species is unlikely.

Up to now, one of the most clinically advanced forms of experimental disease-modifying treatment for Alzheimer's disease is immunization against the Amyloid β protein, which may prevent cognitive impairment. The mechanism is unclear, but a recent paper claimed that anti- Amyloid β antibody can prevent such long-term potentiation inhibition caused by Amyloid β oligomers, suggesting that treatment with such antibodies might rescue reversible cognitive deficits in early Alzheimer's disease (Klyubin et al., 2005) and supporting the involvement of Amyloid β oligomers in Alzheimer's disease cognitive deficit.

Therefore Amyloid β oligomers, also called Amyloid β-derived diffusible ligands (Lambert et al., 1998), have been found guilty of disturbing synaptic function.

How do they cause long-term potentiation impairment and synaptic damage?

First of all, it is crucial to understand where Amyloid β oligomers exert their neurotoxic effect.

Current data show a perineuronal distribution, indicating that oligomers likely accumulate within dendritic arbors (Lacor et al., 2004). Indeed, oligomers extracted from Alzheimer's disease brain were previously found to attach selectively to dendrites in culture (Gong et al., 2003). In particular dendritic targeting of oligomers occurs at synaptic terminals and oligomers, whether synthetic or from Alzheimer's disease brain, colocalize with postsynaptic density protein 95 (PSD-95), a postsynaptic marker found exclusively at synapses in mature hippocampal cultures (Rao et al., 1998).

Regarding the other site of the synapse, presynaptic terminals (labeled for synaptophysin) have larger sizes after 6 h of treatment with Amyloid β-derived diffusible ligands, without changes in the total number of synaptophysin puncta. There are similar indications of enlarged boutons in early Alzheimer's disease (Mukaetova-Ladinska et al., 2000; Scheff and Price, 2003). This pathological enlargement could constitute a homeostatic compensation to preserve synaptic function in presence of oligomers (Lacor et al., 2007).

Therefore, neuronal binding of synthetic and Alzheimer's disease brain-derived Amyloid β-derived diffusible ligands is highly specific (Lacor et al., 2004), despite the potential of Amyloid β for generically inserting into lipid bilayers (Lin et al., 2001) and despite their capability to form large aggregates with other proteins.

These observations suggest that excitatory synapses might be the early targets of soluble Amyloid β, notably the postsynapse, a view supported by evidence that oligomerized Amyloid β can bind PSD-95-containing postsynaptic sites (Lacor et al., 2004) and postsynaptic density complexes containing NMDA receptors (Lacor et al., 2007).

Secondly, which are the mechanisms involved in Amyloid β toxicity exploited at the postsynapsic site?

Several studies have addressed this pivotal question in order to analyse the pathogenic role of Amyloid β oligomers.

Sabatini and coworkers demonstrate that exposure to naturally secreted oligomers of human Amyloid β triggers a reduction of dendritic spine density and a loss of electrophysiologically active synapses in hippocampal pyramidal neurons. In contrast, exposure to Amyloid β monomers obtained from the same cells has no effect on dendritic spine density and oligomer-mediated spine loss is prevented by antibodies against Amyloid β and a small molecule inhibitor of Amyloid β aggregation (Shankar et al., 2007).

The effect on dendritic spines is specific for oligomers which could act like gain-of function ligands that target synaptic spines (Lacor et al., 2007) and disrupt synaptic plasticity (Lambert et al., 1998; Wang et al., 2002). Their cellular actions may be particularly germane to neuropil damage (Klein et al., 2001).

This possibility is supported by findings that Amyloid β-derived diffusible ligands induce abnormal expression of the activity-regulated cytoskeleton-associated protein (Arc) (Lacor et al., 2004), a synaptic memory-related protein, in a manner predicted to cause abnormal spine shape and receptor trafficking (Lacor et al., 2004).

Indeed, recent findings show that Amyloid β-derived diffusible ligands rapidly stimulate loss of critical spine proteins, concomitantly producing aberrant spine morphology and eventually causing a significant reduction in spine abundance. In particular, Amyloid β-derived diffusible ligands promote a rapid decrease in membrane expression of memory-related receptors (NMDA and Ephrin B2 receptors). Moreover, continued exposure results in abnormal spine morphology, with induction of long thin spines reminiscent of the morphology found in mental retardation, deafferentation, and prionoses. Ultimately, Amyloid β-derived diffusible ligands cause a significant decrease in spine density (Lacor et al., 2007).

Moreover, experiments of a recent study indicate that soluble Amyloid β induces a decrease in PSD-95 levels in a time- and dose-dependent manner, without altering the expression of the presynaptic protein synapsin I, the AMPA Glutamate receptor subunit-2 (GluR2), and the kinases Ca^{2+}/Calmodulin-Dependent Protein Kinase II (CaMKII) and cyclin-dependent kinase 5 (cdk5).

The Amyloid β-dependent reduction of synaptic PSD-95, which requires NMDA receptor activity, Ca^{2+} influx, cdk5 activity, and the proteasome pathway, eventually leads to internalization of AMPA receptors. Because AMPA receptors are key players in synaptic function, their surface down-regulation could constitute a crucial mechanism for the synaptic derangement induced by Amyloid β (Roselli et al., 2005).

Another study aimed to explore the mechanism involved in Amyloid β oligomers-induced long-term potentiation inhibition in hippocampal slices.

It is of interest that the very early phase of long-term potentiation, including initial peak amplitude measured at 1 min after High Frequency Stimulation, is inhibited by cell-derived Amyloid β in these *in vitro* studies. This demonstrates that binding of Amyloid β to a substrate affects a very early stage of long-term potentiation induction, i.e. the stimulation of kinases involved in long-term potentiation induction, or early stages of increased AMPA receptor trafficking (Wang et al., 2004). The involvement of various kinases, as Jun N-terminal Kinase (JNK), cdk5 and p38 MAP kinase, in Amyloid β-mediated inhibition of long-term potentiation induction was confirmed by experiments in which Amyloid β was applied in the presence of inhibitors of these kinases (Wang et al., 2004).

Not all the studies focused their attention on long-term potentiation. Malinow and collaborators, in fact, examined the mechanisms underlying Amyloid β-mediated synaptic depression, finding several parallels between long-term depression and Amyloid β-induced synaptic changes. Amyloid β over-expression decreases spine density, partially occludes metabotropic Glutamate Receptor-dependent long-term depression, decreases synaptic AMPA receptor number, and requires second messenger pathways implicated in long-term depression for its depressive effects. Expression of an AMPA receptor mutant that prevents its long-term depression-driven endocytosis blocks the morphological and synaptic depression induced by Amyloid β. Furthermore, Amyloid β can drive phosphorylation of AMPA receptors to a site important for AMPA receptor endocytosis during long-term depression, and mimicking this AMPA receptor phosphorylation produces the morphological and synaptic depression induced by Amyloid β. Taken together, these results show that Amyloid β generates structural and synaptic abnormalities via endocytosis of AMPA receptors (Hsieh et al., 2006).

Despite the differences in the design and analysis, another recent work, supports a model in which Amyloid β perturbs excitatory synapses by enhancing long-term depression in an activity- and calcineurin-dependent manner. Indeed oligomer-mediated spine loss requires activity of a signaling cascade involving NMDA receptors, calcineurin, and cofilin. These results suggest that Amyloid β oligomers shift the activation of NMDA receptor-dependent signaling cascades toward pathways involved in the induction of long-term depression (Shankar et al., 2007).

Furthermore, NMDA receptors internalization occurs through high-affinity binding of Amyloid β_{1-42} to the α7-nicotinic acetylcholine receptor, enhanced α7-mediated Ca^{2+} influx and activation of the serine–threonine protein

phosphatase 2B (PP2B, also known as calcineurin), a Ca^{2+}-sensitive enzyme that regulates NMDA receptor transmission and synaptic plasticity. PP2B dephosphorylates and activates striatal-enriched tyrosine phosphatase (STEP), which dephosphorylates the NMDA receptor subunit 2B (NR2B) at Tyr^{1472} and promotes internalization of NR2B containing NMDA receptors (Snyder et al., 2005). Consistent with this, Amyloid β_{1-42} reduces NMDA excitatory postsynaptic currents in organotypically cultured hippocampal slices (Hsieh et al., 2006).

The findings of these studies, demonstrating that Amyloid β can interfere with postsynaptic function, provide substantial support to the view that Amyloid β may be responsible for the cognitive impairments observed in early-phase Alzheimer's disease patients.

Nevertheless, the regulation of all the proteins mentioned above may constitute a physiological role for Amyloid β peptides, and genetic or other predisposing factors that result in increased production of soluble Amyloid β are likely to trigger pathogenic mechanisms leading to Alzheimer's disease.

5. A vicious circle: synaptic activity influences APP metabolism

In the previous section, we have tried to demonstrate the effects of Amyloid β oligomers on synaptic function. Now, we will deepen the other way round: how synaptic activity can influence APP metabolism.

A variety of circumstantial evidence suggests that, in humans, Amyloid β levels and metabolism may be related in some way to neuronal activity. For example, some patients with substantially elevated neuronal activity, as occurs in temporal lobe epilepsy, develop Amyloid β containing plaques as early as 30 years of age (Gouras et al., 1997; Mackenzie and Miller, 1994). Additionally, the brain regions that develop the highest levels of Amyloid β plaques, such as parts of the frontal and parietal lobes and posterior cingulate cortex, exhibit the highest baseline metabolic activity at rest as measured by positron emission tomography and functional magnetic resonance imaging (Buckner et al., 2005; Gusnard et al., 2001; Raichle et al., 2001). High metabolic activity generally reflects high neuronal and synaptic activity. In addition to human data, there is evidence from animal models and cell culture experiments that links neuronal transport of APP, neuronal activity, and Amyloid β metabolism.

APP is axonally transported from entorhinal cortex to the hippocampal formation via the perforant pathway (Buxbaum et al., 1998); damaging this pathway in APP transgenic mice results in substantially less Amyloid β deposition within the hippocampus (Lazarov et al., 2002; Sheng et al., 2002). Electrical depolarization increases the release of sAPPα in rat hippocampal brain slices (Nitsch et al., 1993). Protein Kinase C as well induces sAPPα production in rat hippocampal slices (Caputi et al., 1997). In studies of both animals and humans, activation of muscarinic M_1 receptors has been reported to increase α-secretase cleavage of APP and to decrease Amyloid β levels (Beach et al., 2001; Hock et al., 2003).

In this frame, neuronal activity plays a causal role in determining the levels of extracellular Amyloid β.

In the last few years, two studies focused on this relevant issue.

Malinow and coworkers demonstrated that neuronal activity modulates the formation and secretion of Amyloid β peptides in hippocampal slice neurons that overexpress APP. In turn, Amyloid β selectively depresses excitatory synaptic transmission onto neurons that overexpress APP, as well as nearby neurons that do not. This depression depends on NMDA receptor activity and can be reversed by blockade of neuronal activity. Synaptic depression from excessive Amyloid β could contribute to cognitive decline during early Alzheimer's disease. In addition, they propose that activity-dependent modulation of endogenous Amyloid β production may normally participate in a negative feedback that could keep neuronal hyperactivity in check. Disruption of this feedback system could contribute to disease progression in Alzheimer's disease (Kamenetz et al., 2003).

More recently, Cirrito et al. performed an *in vivo* study and stimulated the perforant pathway (the major afferent axon route leading from the entorhinal cortex into the hippocampus) while recording electrophysiological activity in the hippocampus and, at the same time, sampling interstitial fluid via microdialysis. Continuous perforant pathway stimulation, which caused epileptiform activity within the hippocampus, caused interstitial fluid Amyloid β to increase by 30% within 1 h, demonstrating a direct relationship between neuronal activity and interstitial fluid Amyloid β.

Conversely, infusion of tetrodotoxin (TTX), which blocks sodium channels and causes a cessation of neuronal activity, significantly decreased basal electrophysiological activity and dropped Amyloid β levels by 40%. Importantly, this effect was reversible, signaling a direct causal relationship between neuronal activity and interstitial fluid Amyloid β levels.

Since synaptic activity increases the concentration of extracellular Amyloid β, the question is still which mechanism is responsible for Amyloid β enhanced concentration. Two possibilities appear likely: first, the half-life of extracellular Amyloid β may be extended; or second, there could be an effect on intracellular APP processing. Using a γ-secretase inhibitor (blocking new generation of Amyloid β) in TTX and vehicle-treated mice, Cirrito et al. showed that Amyloid β half-life was unaltered by depression of neuronal activity. Additionally, expression of the Amyloid β degrading enzyme neprilysin was unchanged in TTX treated mice. Support for the latter possibility (APP processing changes) was provided by Kamenetz et al. (2003). In the Cirrito et al. study, though, TTX-induced reduction in extracellular Amyloid β did not result in changes in CTF99 levels. There was a significant 13% increase in CTF83 level; however, this increase is unlikely to explain the much larger change in Amyloid β levels (Cirrito et al., 2005).

Physiological (and pharmacological) changes in neuronal activity are among the implications of this research. In particular, synaptic activity, may continuously alter the levels of both Amyloid β_{40} and the more amyloidogenic Amyloid β_{42} species

in brain regions prone to formation of amyloid plaques. Thus beyond any fixed genetic influences on APP metabolism or Amyloid β clearance that an individual may harbour, Amyloid β levels may be dynamically regulated by electrical activity.

6. Conclusion

In conclusion, we know that the two main players of Alzheimer's disease pathogenesis are Amyloid β oligomers/peptides and synaptic failure, and that their cross-talk takes place mainly at the postsynaptic site of excitatory synapse. In this scenario, it is fundamental to generate new hypothesis on their interaction, namely to identify molecular linkers between amyloid cascade and synaptic loss associated to the excitatory system. For example it turned out that ADAM10 interacts directly with Synapse-Associated Protein 97 (SAP97), a cargo protein involved in trafficking of glutamate receptors. This interaction is required for ADAM10 localization at post-synaptic membranes and for its enzymatic activity. Moreover, NMDA receptor activation increased SAP97-mediated membrane trafficking of ADAM10 and increased APP cleavage at the α-secretase site (Marcello et al., 2007).

All the reported studies may represent a breakthrough in our comprehension of Alzheimer's disease pathogenesis and add new pieces to the puzzle in the understanding of the complex and coordinated events leading to Alzheimer's disease. As a result, the rapid progress towards the knowledge of the cellular and molecular alterations that are responsible for the neuron's demise may soon help in developing effective preventive and therapeutic strategies.

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