**Bachelor thesis**

# The role of the amyloid-β peptide in Alzheimer's disease

## Mechanisms of Aβ-induced neurotoxicity

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**Summary**

It is generally believed that the amyloid-β peptide plays a central causative role in the pathogenesis of AD. Aβ is present in the brain is several different aggregation forms, ranging from monomers, soluble oligomers to large insoluble fibrillar species. It was first hypothized that the fibrillar species, which are found mainly in extracellular deposits called plaques, are responsible for the neurotoxic effects observed in AD. More recently more attention has been focused on the role of the soluble oligomeric species. However, the question how Aβ induces the toxic events associated with AD and which species are responsible still remains to be solved. Very striking in AD pathogenesis are synaptic signaling impairments and loss of synapses, which shows good correlation with oligomer levels. Aβ induces these synaptic dysfunctions by altering NMDA and AMPA receptor currents by changing activity of several phosphatases and kinases, resulting in deficiencies in LTP and LTD. Further, there are many indications suggestion a role for oxidative stress in association with Aβ peptides. The increased cellular stress results in increased activation of stress proteins and may eventually result in neuronal death. There is also evidence suggesting a role for NGF and its receptors in Aβ induced neuropathology. Results from studies considering the role of Wnt signaling suggest that wnt signaling might be impaired in AD and that its loss of function might be crucial in triggering the neurodegenerative processes induced by Aβ peptides. There is also some evidence which suggests that AD is linked to a state of relative brain insulin resistance, mediated by Aβ. Moreover, addition of Aβ causes failure in several components involved in calcium homeostasis, leading to calcium homeostasis impairments. Further, it has been indicated that Aβ induced mitochondrial failure could be an early event in the pathogenesis of AD. Considering the role of Aβ in cholinergic dysfunction, it has been stated that ACh release and synthesis are depressed and ACh degradation is affected in the presence of Aβ peptides. There is also a large body of evidence supporting the notion that AD is associated with a chronic upregulation of inflammatory responses, induced by Aβ. AD is characterized by progressive loss of neurons, and several mechanisms have been described in which Aβ could lead to apoptosis.

## List of abbreviations used

| | |
|---|---|
| **4-HNE** | 4-hydroxynonenal, a oxidative intermediate |
| **8OHdG** | 8-hydroxyl-2-deoxyguanosine, marker for oxidative stress |
| **8OHG** | 8-hydroxyguanosine, marker for oxidative stress |
| **ABAD** | Aβ binding alcohol dehydrogenase, mitochondrial dehydrogenase |
| **ACh** | Acetylcholine |
| **AChE** | Acetylchonline esterase |
| **AD** | Alzheimer's disease |
| **Akt** | A protein kinase family |
| **AMPAR** | α-amino-3-hydroxy-5-methyl-4-isoxazole receptor, glutamatergic receptor |
| **AP-1** | Transcription factor |
| **APC** | Adenomatous poliposis coli, a scaffold protein |
| **ApoE** | Apolipoprotein E |
| **APP** | Amyloid precursor protein |
| **Aβ** | Amyloid-β |
| **BACE** | β-secretase |
| **Bcl-xl** | B-cell lymphoma-extra large, anti-apoptotic Mitochondrial apoptosis regulating protein |
| **BDNF** | Brain derived neurotrphic factor |
| **Bim** | Member of the Bcl-2 family and a regulator of apoptosis |
| **CaM** | Calmodulin, calcium binding protein |
| **CaMKII** | Ca2+/calmodulin-dependent protein kinase |
| **cAMP** | Cyclic adenine monophosphate |
| **CD36/45/47** | Cluster of differentiation |
| **Cdk** | Cyclin-dependent kinase |
| **ChAT** | Choline acetyltransferase |
| **c-Myc** | Transcription factor |
| **COX** | Cytochrome c oxidase, complex IV in the enzymatic respiratory chain |
| **COX-2** | Cyclooxygenase |
| **CR3/4** | Complement receptor |
| **CREB** | cAMP response element binding, transcription factor |
| **DP5** | Death protein 5 |
| **Dvl** | Disheveled protein, involved in wnt signaling |
| **ER** | Endoplasmic reticulum |
| **Erk** | Extracellular signal-regulated kinase |
| **Fcγ** | Fragment crystallizable region, part of antibody |
| **Fzd** | Frizzled, wnt receptor |
| **GLT-1** | Glutamate transporter |
| **GluR** | Glutamate receptor |
| **GSK3** | Glycogen synthase kinase 3 |
| **HFS** | High-frequency stimulation |
| **Hrk** | see DP5 |
| **IGF-1** | Insulin-like growth factor 1 |
| **IL-1/6/8** | Interleukins |
| **iNOS** | Inducible NOS synthase |
| **IP3** | Inositol triphosphate |
| **JNK** | c-Jun N-terminal kinase |
| **KGDH** | α-ketoglutarate dehydrogenase |
| **LFA-1** | Lymphocyte function-associated antigen 1 |
| **LRP5/6** | Low density lipoprotein receptor-related protein, cell surface receptors |
| **LTD** | Long-term depression |
| **LTP** | Long-term potentiation |
| **MAC** | Membrane attack complex |
| **mAChR** | Muscarinic acetylchonline receptor |
| **MAPK** | Mitogen-activated protein kinase |
| **MCP-1** | Monocyte chemotactic protein-1 |
| **mEPSC** | Miniature excitatory postsynaptic current |
| **MHC II** | Major histocompatibility complex, |
| **nAchR** | Nicotinic acetylcholine receptor |
| **NADPH** | Nicotinamide adenine dinucleotide phosphate |
| **NFT** | Neurofibrillary tangle |
| **NFκB** | Nuclear factor kappa B, transcription factor |
| **NGF** | Nerve growth factor |
| **NMDAR** | N-methyl-D-aspartic acid receptor, glutamatergic receptor |
| **NSAID** | Non-steroid anti inflammatory drugs |
| **p75NTR** | p75 neurotrophin receptor |
| **PDH** | pyruvate dehydrogenase |
| **PDK** | 3-phosphoinositide dependent protein kinase |
| **PI3K** | phosphoinositide 3-kinase |
| **PKA** | Protein kinase A |
| **PKB** | Protein kinase B |
| **PKC** | Protein kinase C |
| **PLAIDD** | p75-like apoptosis inducing death domain |
| **PLC** | Protein lipase C |
| **PP1** | Protein phosphatase-1 |
| **PPAR** | Peroxisome proliferator-activated receptor |
| **PSD** | Postsynaptic density protein |
| **RAGE** | Receptor for advanced glycation endproducts |
| **ROS** | Reactive oxygen species |
| **SAPK** | Stress-activated protein kinase |
| **SEC** | Serpin enzyme complex |
| **SERCA** | Sarcoplasmic/endoplasmic $Ca^{2+}$-ATPase |
| **Smac** | Second mitochondria-derived activator of caspases |
| **SOD** | Superoxide dismutase |
| **SRA** | Scavenger receptor A |
| **Tcf/LEF** | T-cell factor/lymphoid enhancer factor |
| **TNF-α** | Tumor-necrosis factor α |
| **TRAIL** | TNF-related apoptosis inducing ligand |
| **TrkA** | Tropomyosin-related kinase A |
| **VAChT** | Vesicular ACh transporter |
| **Wnt** | Wingless |
| **XIAP** | X chromosome-linked inhibitor-of-apoptosis protein |

## Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder with progressive cognitive decline associated with progressive loss of neurons and synaptic retrogenesis. The risk of AD increases with aging, affecting 7-10% of individuals over age 65 and about 40% over age 80 and in about 50 years it is predicted incidence will increase threefold (Sisodia 1999). Currently, no treatment with a strong disease-modifying is available. Clinically, AD is characterized by global cognitive dysfunction, especially memory impairments and personality changes. AD is characterized by neuropathological hallmarks including neuritic senile plaques, which are extracellular deposits, composed of fibrillar β-amyloid protein (Aβ) and neurofibrillary tangles (NFT) composed of paired helical filaments of hyperphosphorylated tau protein. These histopathological lesions are restricted to particularly the hippocampus and the cerebral cortex, which are involved in memory, reasoning and language. These regions are also reduced in size in patients suffering from AD, as a result from synaptic- and neurodegeneration. Sporadic, which accounts by far for the most cases, and familiar forms of AD are clinically and pathologically indistinguishable, but the familiar forms generally have an earlier onset age (Pereira et al 2005). Although much attention has been focused on the Aβ peptide as (one of the) main causative factors leading to AD, the exact mechanism underlying the pathogenesis of AD has yet to determined. By now there is little debate about Aβ as being one of the players involved in the pathogenesis of AD as there is good evidence indicating that the accumulation of the β-amyloid protein is a primary event in the pathogenesis of AD (Small & McLean 1999). The views on the exact role of Aβ are, however, very diverse. One of the central unanswered questions in AD research is in which way Aβ is toxic to neurons. Which mechanisms are involved, ultimately leading to the cognitive decline associated with AD. Aβ toxicity is a complex phenomenon that may be induced by multiple assembly forms of the Aβ peptide and which can result in a wide variety of effects including reversible synaptic changes but also in neuronal death. In this paper I will provide a summary of the mechanisms in which Aβ could exert its toxic effect on neurons.

**Amyloid-β cascade hypothesis**

β-Amyloid is a 4kDa polypeptide derived by the proteolytic cleavage of the transmembrane amyloid precursor protein (APP) by first β-secretase (BACE) and then γ-secretase (Nunan & Small 2000). The resulting product is a 38 to 43 amino acid long polypeptide. Aβ is present in the brain and cerebrospinal fluid (CSF) of normal humans throughout life (Haass et al 1992). Normally, the main excreted form of Aβ has 40 residues, but in (familial) AD brain the carboxyl extended, readily aggregating, $A\beta_{42}$, is increased and it is believed that this form is responsible for the toxic effects (Citron et al 1997).

There are seven major pieces of evidence supporting the notion of a causative role for Aβ. First, AD like neuropathology is invariably seen in Down's syndrome patients. This results from increased APP expression and consequent higher Aβ levels as expected by the localization of the APP gene to chromosome 21 (Prasher et al 1998). Second, Aβ peptides are toxic to cortical and hippocampal neurons (Deshpande et al 2006). Third, mutations in or near the Aβ region in the APP gene alter the amount or aggregation properties of Aβ and are sufficient to cause early-onset AD (Levy et al 1990). Fourth, mutations in presenilins, which constitutes the catalytic site of γ-secretase increase $A\beta_{42}$/ $A\beta_{40}$ ratio and cause very early forms of AD (Kumar-Singh et al 2006). Fifth, Apolipoprotein E (ApoE) ε4, a major risk factor for late-onset AD, transgenic mice show increased Aβ fibrillogenesis (Fagan et al 2002). Sixth, human APP transgenic mice show an increase in extracellular Aβ and neuropathological and behavioral changes similar to those seen in AD (Ashe 2005). Finally, injection of Aβ or co-expression of human APP in tau transgenic mice increases tangle formation (Gotz et al 2001; Lewis et al 2001).

According to the amyloid cascade hypothesis, the accumulation of Aβ fibrils resulting from an imbalance between production and clearance is the initiating molecular event that also triggers the downstream neuropathologic, e.g. NFT formation, conditions in AD (Hardy & Selkoe 2002). Several observations suggest that tau hyperphosphorylation is a downstream aspect induced by Aβ (Gotz et al 2001; Hutton et al 1998).

Recent data led to a modified version, the Aβ cascade hypothesis. According to this hypothesis, other, less well characterized, soluble, non-fibrillar species of Aβ may be responsible for the cognitive impairments in AD and this hypothesis has gained increased interest over the past few years and is greatly supported by many experimental findings (Walsh & Selkoe 2007). In human brain it has long been recognized that senile plaque numbers has a weak correlation with severity of cognitive decline (Terry et al 1991). However, soluble Aβ levels correlate well with synaptic loss and severity of dementia (McLean et al 1999). Moreover, memory impairment and changes in neuron function in APP transgenic mice occur before amyloid deposition (Chapman et al 1999). Further, recent advances in neuroimaging techniques have shown the presence of robust plaques in non-demented people (Villemagne et al 2008). Walsh and colleagues showed for the first time

that a low-n oligomeric (dimers, trimers, tetramers) assembly of naturally secreted human Aβ alters hippocampal synaptic plasticity (Walsh et al 2002). Also, the number of spines dramatically decreases when neurons were incubated with Aβ oligomers but not with monomers. The decrease in spine density could be reversed when neurons where treated with an anti-Aβ antibody (Shankar et al 2007). Oligomeric Aβ inhibited neuronal viability 10 fold more than fibrillar Aβ and 40 fold more than monomeric Aβ (Dahlgren et al 2002). The Aβ cascade hypothesis has also left room for the role of intracellular Aβ accumulation in vulnerable brain regions, in which it might be an early event in the pathogenesis of AD (Wirths et al 2004). Cell biological studies reported that Aβ generation occurs at the ER, the Golgi apparatus (Hartmann et al 1997) and the endosomal-lysosomal system (Pasternak et al 2004). Further, it has been shown that $A\beta_{42}$ aggregates into oligomers within endosomal vesicles and along microtubules of neuronal processes (Takahashi et al 2004). It has also been described that extracellular Aβ could be internalized by neurons, after which it might induce noticeable up regulation of newly generated Aβ peptides, as well as trigger the toxic effects associated with Aβ (Glabe 2001), which are further described below. Schmitz and colleagues presented the first evidence that neurodegeneration correlated with intraneuronal Aβ accumulation rather than extracellular plaque formation (Schmitz et al 2004). Moreover, it has been suggested that the extracellular plaques could be the consequence of lysis of Aβ burdened neurons (D'Andrea et al 2001), or even may be benign and protective in nature (Caughey & Lansbury 2003).

The role of Aβ in pathogenesis of AD and the identification of the responsible species have yet to be determined. One recent proposal states that an increase in the $A\beta_{42}$/ $A\beta_{40}$ ratio, rather than the absolute levels of $A\beta_{42}$, triggers the deleterious events leading to AD (Pimplikar 2009). It is reasonable to suggest that different Aβ aggregates may have different pathological effects, which results in neuronal death and synaptic degradation, because much research on the role of Aβ, is performed with different Aβ species, and shows a more or less contrary result. For example, Shankar and colleagues have reported the presence of Aβ dimeric and trimeric species in AD brain but not in normal brain and that these particular species are neurotoxic whereas the higher oligomeric or fibrillar species exert no toxicity (Shankar et al 2008). In contrast, another set of studies showed that neurotoxicity was associated with larger aggregates termed Aβ*56, possibly composed of 12 monomers (Lesne et al 2006). Aβ oligomerization and fibrillization may result from independent and distinct aggregation mechanisms, because inhibitors of aggregation can be divided in three classes; some inhibit fibrillization, some inhibit oligomerization and some inhibit both (Necula et al 2007). The nature of the neurotoxic Aβ species is very difficult to define, because monomers, soluble oligomers, insoluble oligomers and amyloid fibrils are believed to exist in dynamic equilibrium in the brain.

It should be noted that the amyloid/Aβ cascade hypothesis is not universally accepted in the field. The existing supporting data might not be as strongly supportive as initially perceived and the accumulation of inconsistent data with the main tenets of the hypothesis is a reason

for this. It is not surprising that Aβ is toxic to neurons considering the amphiphilic, detergent-like properties of Aβ. Therefore, it is more relevant to ask whether Aβ is toxic in this manner in AD. Aβ is not free inside the brain, but bound to other proteins because of its hydrophobic nature, and the bound proteins might also account for at least some of the pathogenic conditions in AD brain. There are some studies which suggest that Aβ is only toxic when it is in conjunction with other factors such as metal ions, oxidative stress or excess of excitatory amino acids (Morgan et al 2004). Further, there are approximately 225 different mutations in presenilin genes and APP gene that all initiate familiar AD at different stages. The molecular outcome however, differs over mutations; some increase total Aβ, some do not affect Aβ, some increase $A\beta_{42}$ while others decrease $A\beta_{40}$ (Van Broeck et al 2007). It is rather surprisingly that all those mutations have the same pathologic outcome. There is also a substantial body of evidence indicating that mutations in presenilin 1 can trigger AD independent of Aβ or APP (Shen & Kelleher 2007). Moreover, animal models of AD fail to produce NFTs and might therefore be considered as models for amyloidopathy rather than for AD (Radde et al 2008).

At one side of the spectrum there are those who consider a yet to determined species of Aβ to be the cause of AD, on the other hand there are also some who suggest the Aβ based hypotheses are wrong. It has even been suggested that Aβ may be benign in nature (Lee et al 2007). From the discussion above it should become clear that the amyloid/Aβ cascade hypothesis is the best defined and accepted view, although the evidence that Aβ is the cause for AD is not as strong as believed by some proponents. Therefore, it may be helpful to reassess the amyloid/Aβ cascade hypothesis from the classical view that Aβ lies upstream of the other deleterious events associated with AD (fig. 1A), to a view in which Aβ lies at the same level of the other events that can also be caused by non-Aβ factors (fig. 1B). One such view places ApoE upstream of both Aβ accumulation and tau hyperphosphorylation (Tiraboschi et al 2004). The dominant view is that ApoE ε4, released from astroglia, increases levels of Aβ by decreasing its clearance (Bales et al 2002). It is proposed that ApoE also influences tau hyperphosphorylation by increasing phosphorylation of tau by glycogen synthase kinase 3 (GSK-3) by binding to tau (Gibb et al 2000), or by binding to cell surface LDL and LDL receptor-related proteins (LRPs) (Herz & Bock 2002). GSK-3 activity is directly regulated by LRP5 and LRP6 which both can bind to ApoE. As a result, the binding of wnt, which normally reduces GSK-3 activity, to LRP5 and LRP6 is prevented, which in turn leads to increased kinase activity and tau hyperphosphorylation (Caruso et al 2006). Further, GSK-3 might increase Aβ production by affecting enzymatic APP processing (Phiel et al 2003). A second upstream factor influencing both Aβ production and NFT formation is the retromer-binding receptor sorLA. SorLA binds to APP, thereby increasing interaction with its cleavage enzymes which could lead to increased Aβ production (Small & Gandy 2006). SorLA deficiencies could lead to NFT formation by a mechanism which involves wnt signaling, but in a different manner than ApoE; wnt is transported out of the cell after translation by the WLS chaperone, WLS is then transported back by sorLA (Belenkaya et al 2008). Thus, a deficiency

in sorLA could lead to decreased wnt signaling via LRP5 and LRP6, leading to increased GSK-3 activity and NFT formation. There is more about wnt signaling in AD under '*The role of wnt signaling*' further below.

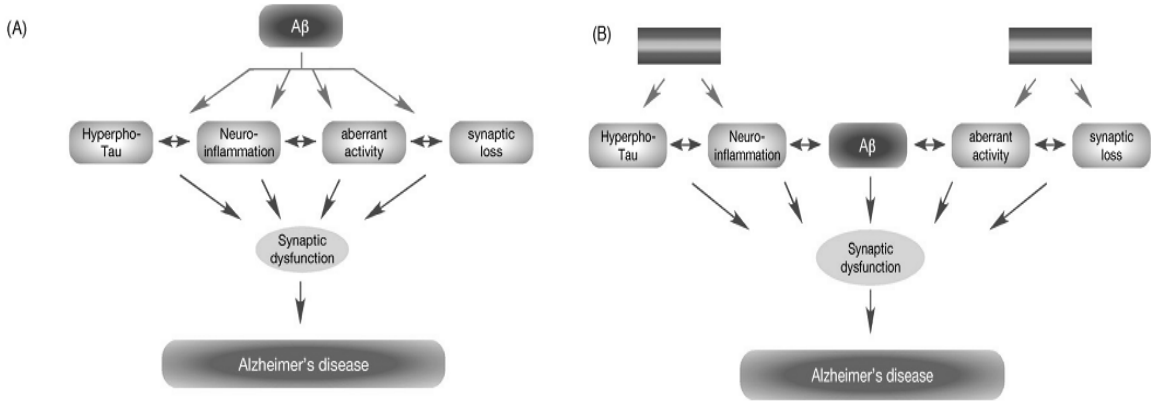


Figure 1. (A) The classical view on the role of Aβ in AD. (B) The reassessed view on Aβ as proposed by Pimplikar, the upper gray boxes are a yet to be determined upstream factor leading to AD pathology. Figures adopted from Pimpiklar et al 2009.

Another well supported hypothesis focuses on a causative role for the microtubule binding protein tau, which forms NFTs in AD. NFTs are formed of hyperphosphorylated tau monomers. The use of Aβ reducing pharmaceuticals has been largely disappointing in reducing the cognitive impairment in AD to date (Small & Duff 2008). Nonetheless it is easy to defend the amyloid cascade hypothesis by invoking the plausible assumption that Aβ can act as a trigger of downstream events such as NFTs, and once initiated the disease progresses independent of Aβ levels. This claim is supported by behavioral research in which a single injection of $A\beta_{42}$ aggregates was sufficient to induce behavioral changes which worsened over weeks (O'Hare et al 1999). Several mechanisms have been elucidated which lead to NFT formation. In vivo studies have demonstrated that GSK-3 and cyclin dependent kinase 5 (Cdk5) can phosphorylate tau (Noble et al 2003; Spittaels et al 2000) and are probably involved in AD. Cdk5 is bound to p35, which is cleaved by calpains to p25 after insults like oxidative stress, inflammation and excitotoxicity and the Cdk5/p25 complex is more stable and is thought to be responsible for tau phosphorylation. Further, tau can be dephophorylated by protein phosphatase-1 (PP1), which is decreased by Cdk5 by activating PP1 inhibitors (Shelton & Johnson 2004). These mechanisms seem to be independent of Aβ, it is however, not impossible that Aβ also could influence these mechanisms indirectly in a yet to be elucidated way.

**Effects of Aβ on synaptic dysfunction**

The memory impairments and cognitive decline observed in AD patients, correlates better with synaptic dysfunction than plaque or tangle formation. Loss of synapses without an associated loss of neurons occurs well before plaque deposition is observed (Selkoe 2002). This could indicate a role for soluble oligomeric species of Aβ. Indeed, correlations between soluble Aβ levels in the brain and synaptic loss were observed (McLean et al 1999) and a single intraperitoneal injection with Aβ antibodies reversed the memory deficits in APP transgenic mice without affecting amyloid plaque burden (Dodart et al 2002). Further, Aβ oligomers seem to target and disrupt synapses in AD brain sections (Lacor et al 2004). The main site for oligomeric Aβ accumulation seems to be the excitatory synapses, which could be due to copper and zinc release during synaptic transmission since the copper and zinc binding 8-OH-quinoline clioquinol reduced oligomeric Aβ accumulation at these synapses (Deshpande et al 2009). There are, however, also recent reports which indicate a detrimental role of fibrillar Aβ on synaptic function, decreasing their ability to integrate and propagate information (Tsai et al 2004). Protofibrillar Aβ species can alter membrane excitability by a mechanism involving inhibition of specific $K^+$ currents and glutamate receptors (Ye et al 2004). Below I will discuss these aspects in more detail.

Long-term potentiation (LTP) increases communication between two neurons by enhancing synaptic transmission between excitatory glutamate synapses. LTP is induced by coincident presynaptic glutamate release and postsynaptic depolarization, resulting in postsynaptic calcium influx through N-methyl D-aspartate (NMDA) receptors, a class of ligand gated ionotropic glutamate receptors which mediate rapid glutamatergic synaptic transmission. The postsynaptic calcium influx activates an intracellular signaling cascade that includes several kinases and lead to increased numbers of postsynaptic glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazole (AMPA) receptors. In contrast, long-term depression (LTD) requires a lower rise in calcium concentration and involves removal of AMPA receptors (Malinow & Malenka 2002). Animal models of AD show disruption of excitatory synaptic transmission and LTP (Rowan et al 2003). Walsh and Selkoe concluded that low-n oligomers of human Aβ at picomolar concentrations could potently inhibit the maintenance of LTP in the hippocampus and that trimers are even more potent than dimers (Selkoe 2008; Townsend et al 2006). LTP is inhibited after high-frequency stimulation 40 fold by $Aβ_{42}$ as compared to $Aβ_{40}$(Rowan et al 2004). Especially the early phase LTP, including peak amplitude, was reduced by Aβ, indicating that Aβ can regulate early processes necessary for LTP induction, such as the dysregulation of kinases and phosphatases.

Inhibition of the phosphatases calcineurin and PP1 prevented Aβ induced LTP deficits (Chen et al 2002; Knobloch et al 2007), suggesting that altered activation of these phosphatases is a key event in Aβ induced LTP deficits. Calcineurin activity is calcium dependent and it has been shown to induce LTD via PP1 (Mulkey et al 1994). Picomolar levels of Aβ reduce calcium influx through NMDA receptors (Selkoe 2008). Calcineurin inhibition also prevented

dentritic spine loss, further supporting a role for calcineurin in AD (Shankar et al 2007). More importantly, this suggests that Aβ induces a shift to LTD in LTP/LTD balance rather than a block of LTP. Chen and colleagues have proposed that Aβ influences different stages of LTP, specifically the early and late components (Chen et al 2002). $A\beta_{42}$ application prior to high frequency stimulation (HFS), which triggers LTP, immediately attenuates LTP and NMDA receptor currents, while application minutes after HFS caused little attenuation in early-phase LTP but inhibited late-phase LTP by inhibition of protein synthesis. This is further confirmed by administration of emetine, a protein synthesis inhibitor, after which a similar decay in late-phase LTP is observed. Administration of Aβ and emetine together did not further attenuate this. A possible mechanism by which Aβ inhibits protein synthesis is by calcineurin. There are two ways in which calcineurin might influence late-phase LTP; the first mechanism is conductance of LTP signals through the NMDA receptor, which works through a calcineurin-dependant mechanism, another explanation is inhibition of protein synthesis. Protein synthesis in late-phase LTP is initiated by phosphorylation of CREB (cyclic adenine monophosphate (cAMP) response element binding) protein, which in turn is deactivated by dephosphorylation by calcineurin, which suggests that Aβ works by enhancing CREB dephosphorylation by calcineurin, possibly via increased activity of PP1, which is positively regulated by calcineurin and negatively by protein kinase A (PKA) (Knobloch et al 2007; Yamin 2009) (fig 2).

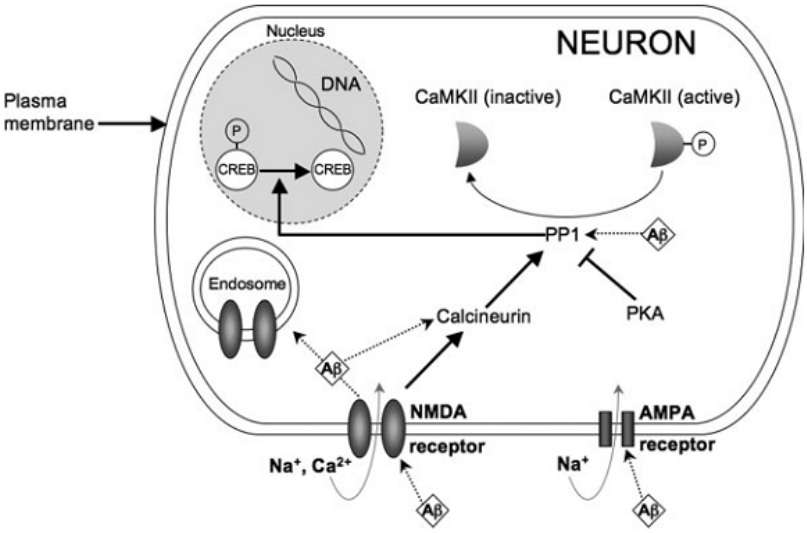


Figure 2. Aβ induced disruptions in NMDA receptor-dependent pathways that underlie LTP deficits. Figure adopted from Yamin 2009.

The kinases Cdk5, c-Jun N-terminal kinase (JNK), GSK-3 and p38 mitogen activated protein kinase (MAPK) show increased activation after Aβ treatment which could account for the block in LTP (Koh et al 2008; Wang et al 2004). The activity of the kinases extracellular signal-regulated kinase (Erk)/MAPK, Akt/protein kinase B (PKB) and calcium/calmodulin-dependent protein kinase II (CaMKII) is blocked by Aβ which could be responsible for the LTP block

(Selkoe 2008; Townsend et al 2007). Data on PKA are inconsistent; Selkoe found no change in PKA activity whereas Vitolo found a reduced activity of PKA and subsequent CREB which might be responsible for the impairment of synaptic plasticity (Vitolo et al 2002). A possible upstream mediator of the disruption of the activity of some of these kinases might be the insulin receptor, because insulin receptor antagonists mimic the effect of Aβ on LTP and the activation of these kinases. This is restored by treatment with insulin (Townsend et al 2007). Another study, by Zhao and colleagues, downstream of the NMDA receptor has focused on oligomeric Aβ and CaMKII, of which disruption is presumed to be critical during synaptic plasticity by influencing the dynamic balance between phosphatases and kinases at the postsynaptic sites. It was found that after Aβ treatment CaMKII levels remained constant, but the phosphorylated fraction was lower. A known phosphorylation target of CaMKII, $Ser^{831}$ on GluR1 receptors on AMPA receptors, was also studied. After HFS, samples treated with Aβ showed no difference with non-stimulated, Aβ treated control samples in $ser^{831}$ phosphorylation (Zhao et al 2004). This suggests that blocking CaMKII activity-dependent phosphorylation may be important in Aβ induced LTP deficits. In additional research it was found that distribution of CaMKII in cortical neurons is altered as an effect of Aβ treatment; the synaptic pool is reduced while the cytosolic pool is increased (Gu et al 2009). How Aβ affects CaMKII translocation is unknown, but it seems likely that intracellular calcium signaling and/or actin cytoskeleton dynamics are involved, because it has been shown that CaMKII distribution depends on $Ca^{2+}$/CaM (calmodulin)(Shen & Meyer 1999) and F-actin (Shen et al 1998).

Since Aβ is very hydrophobic and has been shown to bind many cell surface proteins and receptors (Verdier & Penke 2004) it has been suggested that Aβ directly binds to and modulates AMPA receptor channel properties. Indeed, in neurons from the hippocampal CA1 region iontrophoretically applied $Aβ_{42}$ attenuated AMPA-evoked neuronal firing whereas NMDA-evoked firing were potentiated (Szegedi et al 2005). Even a mild initial abnormal NMDA receptor functioning, either by Aβ or by glutamate, could cause neuronal death by initiating cyclic neurotoxic effects in which a shift from α-secretase to β-secretase results in excessive Aβ production and further glutamate accumulation (Lesne et al 2005; Parameshwaran et al 2008). Aβ oligomers appear to bind to NMDA receptors at the synapse and trigger NMDA receptor internalization and deregulation of NMDA signaling pathways (Shankar et al 2007), as described above.

There is evidence that Aβ induces a chemical LTD which is manifested as a depression in AMPA- and NMDA receptor mediated signaling (Hsieh et al 2006). This depression is possibly due to a reduction in cell surface expression of AMPA and NMDA receptors. Aβ can reduce NMDA receptor surface expression quickly via activation of nicotinic acetylcholine receptors (nAchRs) (Snyder et al 2005), while reduction in AMPA receptor requires constant Aβ exposure over days (Hsieh et al 2006). In oligomer treated neurons miniature excitatory postsynaptic current (mEPSC), which reflect the postsynaptic AMPA receptor mediated response to the release of a single vesicle of glutamate, is reduced, indicating a loss of

excitatory synapses (Shankar et al 2007), Parameshwaran found that this was only the case with $A\beta_{42}$ and not with $A\beta_{40}$ (Parameshwaran et al 2007). It is possible that the structural alterations of the synapse are a consequence of the removal of glutamate receptors and postsynaptic density protein (PSD)-95 from the synapse. Aβ reduces CaMKII and PSD-95, which are both essential for AMPA receptor anchoring to the synapse and maintenance of the postsynaptic spine structure (De Roo et al 2008). These effect are similar to those observed in a developing brain in which activity-dependent AMPA receptor silent synapses are generated (Wasling et al 2009). Synaptic retrogenesis occurs because the AMPA receptor silent synapses cannot be un-silenced by LTP, which brings back a set of AMPA receptors in a developing brain. These synapses are therefore bound to be eliminated (Calabrese et al 2007) (fig. 3). A study by Shankar and colleagues suggests that spine retraction is NMDA receptor dependent, because the NMDA receptor antagonist CPP completely prevented spine loss after incubation with Aβ (Shankar et al 2007).

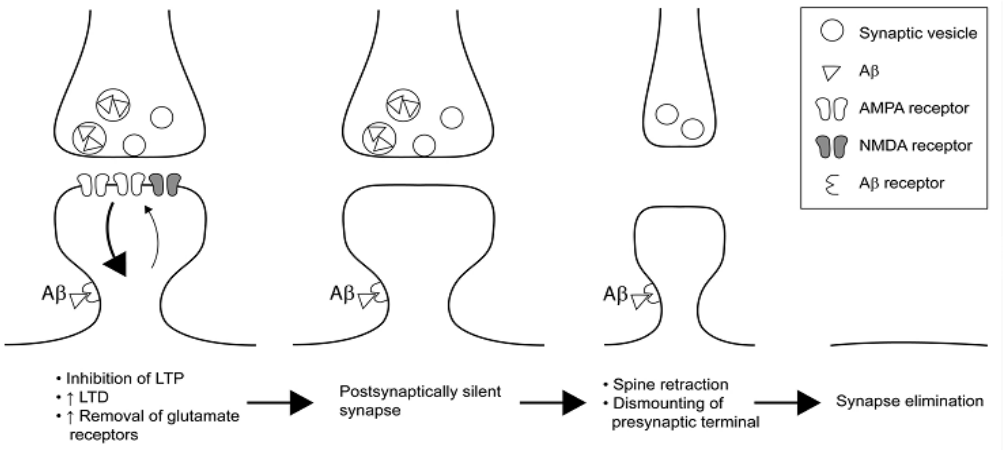


Figure 3. Proposed model of Aβ induced synaptotoxic effects and synapse elimination in AD. Figure adopted from Wasling et al 2009.

Microglial cells, the immune effector cells of the brain, surround Aβ plaques in AD brain (McGeer & McGeer 1995) and Aβ activates microglia in culture (Tan et al 1999). Increased activity of p38MAPK and JNK is known to be associated with microglial activation in AD (Hensley et al 1999; Zhu et al 2004). Aβ failed to inhibit LTP induction after pretreatment with inhibitors of these kinases or after treatment with minocycline, a rapid and selective inhibitor of microglial activation (Rowan et al 2004). Moreover, inhibition of the inducible NO synthase (iNOS) in microglia prevented the inhibition by Aβ on LTP, which is consistent with data from iNOS knock-out mice. This suggests a role for the peroxynitrite free radical, which is formed after NO reaction with superoxide anion. This is supported by data obtained from experiments in which the antioxidative enzymes superoxide dismutase (SOD) and catalase were simultaneously added, in this experiments Aβ induced LTP inhibition was prevented (Rowan et al 2004). This leads us to a possible role for the brain immune system and oxidative stress associated with Aβ.

## Aβ induced oxidative stress

It is currently believed that oxidative stress has a significant role in pathogenesis associated with Aβ peptides. In AD brain, DNA and RNA oxidation is marked by increased levels of 8-hydroxyl-2-deoxyguanosine (8OHdG) and 8-hydroxyguanosine (8OHG) (Mecocci et al 1997; Nunomura et al 1999). Further, increased levels of protein carbonyl and nitration of tyrosine residues are found, which is indicative of elevated oxidative modification of proteins (Smith et al 1996). Protein oxidation is only observed in brain region where $A\beta_{42}$ is present, and not in e.g. the cerebellum, which is largely spared by AD (Hensley et al 1995). Furthermore, there is also indirect evidence from studies showing that treatment with antioxidants, e.g. vitamin E (tocopherol, TCP) delays the progression of AD (Sano et al 1997). It has been shown that, in an animal model of AD, an increased load of reactive oxygen species (ROS) is associated with plaques (McLellan et al 2003). Aβ binds $Cu^{2+}$ during aggregation with high affinity and reduces $Cu^{2+}$ to $Cu^{+}$, creating a complex with strong reducing potential which mediates the reduction of $O_2$ to $O_2^{-}$, subsequently resulting in $H_2O_2$ generation (Opazo et al 2002). Further, it has been found that Aβ induces lipid peroxidation and subsequent 4-hydroxynonenal (4-HNE) production, a cytotoxic aldehyde, which could lead to increased vulnerability to apoptosis via JNKs and P38MAPK (Tamagno et al 2003). Further, 4-HNE impairs glucose and glutamate transport and induces mitochondrial oxidative stress and dysfunction (Keller et al 1997). For example, 4-HNE binds to the glial glutamate transporter GLT-1, which could explain its loss of function in AD (Lauderback et al 2001). Another way in which oxidative stress might play a role in Aβ induced neurotoxicity is by binding copper. After binding, copper is reduced by Aβ and the formed complex potentiates $H_2O_2$ formation (Huang et al 1999). Yet another way in which Aβ induces ROS formation is by binding of fibrillar Aβ to the receptor for advanced glycation endproducts (RAGE), which initiates an oxidative inflammatory response (Yan et al 1996). Microglial cells also produce free radicals, once activated by Aβ plaques, but this is described in more detail under '*Neuroinflammation*.'

In AD, the stress-activated protein kinase (SAPK)/JNK pathway is altered. SAPKs are central mediators that propagate stress signals from the membrane to the nucleus, either leading to neuronal cell death or activation of protective mechanisms, depending on cellular and environmental conditions as well as interaction with other signaling pathways (Mielke & Herdegen 2000). Apparently, SAPK/JNK activation precedes Aβ deposition, which makes it plausible that Aβ initially does not activate SAPK/JNK, although it may activate it later on (Zhu et al 2001). It has been shown that Aβ induces a two to threefold increase in JNK/SAPK activation and that this directly contributes to Aβ induced neuronal cell death (Troy et al 2001). A feed-forward cycle has also been proposed, in which Aβ or oxidative stress activates JNK/SAPK, which mediates BACE activation and consequent Aβ production (Tamagno et al 2005).

It has been shown that methionine residue 35 of $A\beta_{42}$ is involved in its toxicity because substitution of the S atom with methylene ($CH_2$) completely abolished oxidative and

neurotoxic properties (Yatin et al 1999). Oxidation of the methionine residue supports the hypothesis that oligomeric forms are the toxic species and not per se the fibrillar ones, because these oxidized, hydrophobic, oligomers could be inserted into neuronal lipid bilayer to induce lipid peroxidation and subsequent 4-HNE formation (Butterfield 2002).

Aβ peptides have been proposed both as a source and a consequence of oxidative stress. Oxidative balance is tightly regulated, therefore it is expected that compensatory mechanisms are upregulated in AD. Indeed, several reports have shown that this could be the case, but it is beyond the scope of this paper to describe it further. Aksenov and colleagues performed a study to the expression of several antioxidative enzymes in AD brain regions (Aksenov et al 1998). More interesting is the notion that Aβ deposits have an inverse correlation with 8OHG and that oxidative stress precedes plaque formation (Nunomura et al 2000). This might suggest that Aβ production represents a cellular response to elevated oxidative stress and serve in an antioxidant function.

**The role of neurotrophin mediated neurotoxicity**

Oxidative stress also seems to play a role in neurotrophin mediated neuronal toxicity induced by Aβ. Receptor levels of tropomyosin-related kinase A (TrkA) and the p75 neurotrophin receptor ($p75^{NTR}$), which are both receptors for the neurotrophin, nerve growth factor (NGF), are downregulated in neuronal cells after Aβ treatment. Further, these cells also secrete more NGF. Initially, receptor levels of $p75^{NTR}$ increase, which indicates that vesicular stores of $p75^{NTR}$ fuse to the plasma membrane (Olivieri et al 2002). Similar results were found after treating the same cells with $H_2O_2$, which indicates a role for oxidative stress. Contrary, another very recent study reports that in cells under apoptotic Aβ exposure the following events occur: I. NGF and TrkA gene expression is upregulated, which increases TrkA protein and NGF secretion; II. TrkA/Akt/GSK-3β signalling is activated (Bulbarelli et al 2009). Nevertheless, TrkA phosphorylation is not completely abolished after preventing NGF/TrkA binding, suggesting that Aβ contributes to TrkA activation by a different mechanism, possibly via a direct interaction of Aβ with the plasma membrane (Kayed et al 2004). Together with the fact that membrane perturbations lead to auto-phosphorylation of TrkA (Mutoh et al 1995), this opens the possibility of a direct effect of Aβ on membrane perturbation, leading to increased TrkA activation. Yaar and colleagues have shown that Aβ binds to $p75^{NTR}$ to form the Aβ-$p75^{NTR}$ complex, which contains either monomeric $p75^{NTR}$ or trimeric $p75^{NTR}$ (Yaar et al 2002). The binding sites of NGF and Aβ on $p75^{NTR}$ are distinct (Susen & Blochl 2005), and it has been suggested that the amino acids in the 29-35 region of Aβ are crucial for the effects mediated through $p75^{NTR}$ (Coulson 2006). An alternative explanation is that Aβ associates with a component of the γ-secretase complex, thereby modulating γ-secretase cleavage of $p75^{NTR}$ and influencing its signal transduction, without necessarily binding direct to $p75^{NTR}$ (Jung et al 2003; Kanning et al 2003). Aβ triggers the downstream components JNK, G-proteins, nuclear factor κB (NFκB) and phosphoinositide 3-kinase (PI3K). The death domain region of $p75^{NTR}$ phosphorylates JNK (and also G-proteins and NFκB), but its cascade and cell death are promoted more efficiently when coupled with the chopper domain. The chopper domain alone does not activate JNK, but initiates the early death promoting signals mediated through a mitochondrial-dependent apoptotic pathway (Coulson 2006). In addition, brain-derived neurotrophin factor (BDNF) and CREB are downregulated by oligomeric Aβ treatment of cortical neurons (Garzon & Fahnestock 2007). In contrast, Olivieri and colleagues found that Aβ treatment upregulates TrkB and BDNF, which is reverted by antioxidant treatment (Olivieri et al 2003). It should be noted that BDNF regulation is maintained through cholinergic innervation and through NMDA receptors (da Penha Berzaghi et al 1993; Thoenen et al 1991), which opens the possibility that dysfunction of the cholinergic or glutamatergic system might be an upstream factor.

**Aβ and wnt signaling**

Wnt signaling plays a crucial role in cell fate determination and adhesion during development. Wnt genes encode a secreted glycoprotein Wnt ligand (350-400 AAs), which binds to the transmembrane receptor frizzled (Fzd). In the canonical pathway Fzd transduces the signal to the intracellular space by activating disheveled (Dvl) protein, which in turn inhibits GSK-3β through binding scaffold proteins axin and adenomatous poliposis coli (APC). Active GSK-3β phosphorylates β-catenin for ubiquitin-proteasome-mediated degradation (Aberle et al 1997). As a result of GSK-3β inactivation, intracellular β-catenin levels will increase, allowing it to bind the transcription factors T-cell factor/lymphoid enhancer factor (Tcf/LEF). The resulting β-catenin-Tcf/LEF complex activates the expression of wnt target genes (Nelson & Nusse 2004) (fig 4). The noncanonical wnt pathway does not influence β-catenin stability and β-catenin mediated gene expression.

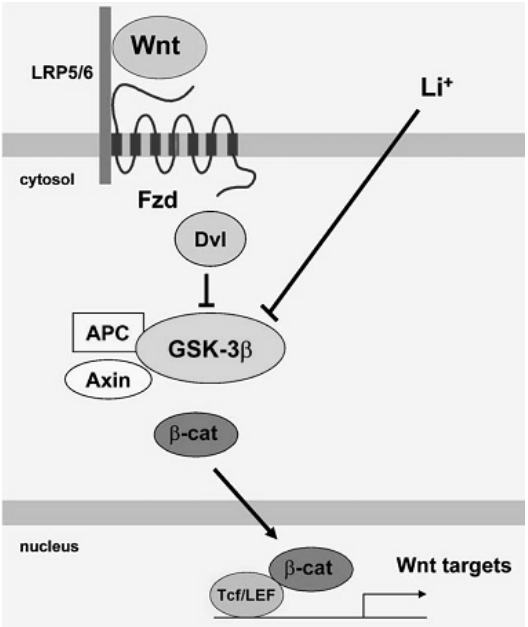


Figure 4. The wnt/β-catenin pathway. Figure adopted from Fuentealba et al. 2004.

In AD, wnt signaling has been placed as a core pathway, linking both plaque and NFT pathology because wnt signaling interacts with GSK -3β and might influence APP processing (De Ferrari & Inestrosa 2000). In animal models, it has been shown that in the hippocampus both β-catenin decrease and GSK -3β activation correlate with tangle pathology and neurodegeneration (Lucas et al 2001) and it has been suggested that β-catenin mediated transcription prevents Aβ induced neurotoxicity (Zhang et al 1998). More recently Fuentealba and colleagues found that after exposure to Aβ fibrils, almost no cytoplasmatic β-catenin is present (Fuentealba et al 2004). Further, wnt target gene expression correlates with cytoplasmatic β-catenin levels modulated by Aβ (decrease) or the canonical wnt-3a ligand (increase) (Alvarez et al 2004). In addition, stimulating the noncanonical wnt pathway

by wnt-5a ligand prevents the decrease in PSD-95 and NMDA receptors in hippocampal neurons (Dinamarca et al 2008). These results suggest that in AD, wnt signaling might be impaired and that its loss of function may play a crucial role in triggering the neurodegeneration induced by Aβ. Failure in wnt signaling could possibly induce a vicious circle, in which first APP processing is altered, after which intracellular Aβ deposition occurs. This intracellular Aβ could influence many signaling and metabolic pathways which in turn could result in cell death and extracellular deposition of Aβ, further enhancing Aβ induced toxicity which could eventually lead to AD (De Ferrari & Inestrosa 2000).

**The role of insulin signaling and cholesterol in Aβ mediated toxicity**

There is evidence that AD is linked to a state of relative brain insulin resistance, also called type III diabetes (de la Monte et al 2006). In healthy brain, insulin and insulin-like growth factor 1 (IGF-1) promote glucose utilization and neuronal survival mainly through PI3K/Akt/GSK-3β signaling and is vital for neuronal metabolism and survival (Bondy & Cheng 2004). In AD brain, however, levels of insulin and IGF-1 are dysregulated (Moloney et al 2008). Intracellular expression of Aβ leads to a decrease in phosphorylated Akt levels, an increase in activated GSK-3β and induction of apoptosis (Magrane et al 2005). There is recent evidence that intracellular Aβ interrupts insulin signaling by inhibiting 3-phosphoinositide dependent protein kinase (PDK) activity, via interference of binding to its target, Akt (Lee et al 2009). It has further been recognized that Akt deactivation is a mediator for oxidative and excitotoxic neuronal death (Luo et al 2003). Moreover, Akt phosphorylates GSK-3β which results in increased glycogen synthesis. Several reports also show a role for the extracellular pool of Aβ. Application of soluble Aβ prevents insulin from binding to its receptor (Xie et al 2002) and causes loss of surface expression of insulin receptors (Townsend et al 2007).

Cholesterol is very abundant in neurons and glial cells, and is essential for formation and maintenance of cell membranes. It has been shown that cholesterol is essential for Aβ binding to cell membranes and cytotoxicity (Subasinghe et al 2003). It was also reported that cholesterol could be oxidized by Aβ, leading to 7β-hydroxycholesterol which might be neurotoxic as a proapoptotic oxycholesterol (Nelson & Alkon 2005). Another mechanism by which Aβ and cholesterol are detrimental, is by cholesterol release induced by Aβ and subsequent forming of the Aβ-HDL complex, which could not be internalized, leading to a decrease in cellular cholesterol availability (Michikawa 2003).

**Effects of Aβ on calcium homeostasis**

Calcium signals are important for neurons, they control membrane excitability, trigger neurotransmitter release, mediate activity-dependent changes in gene expression and modulate neuronal differentiation and transition to apoptosis (Berridge et al 1998). Tangle-bearing neurons show increased amounts of calcium and increased levels of calcium-dependent proteases and calcium activated kinases, pointing to a possible role of calcium balance in AD pathogenesis (Grynspan et al 1997). In areas close to plaques, increased basal calcium levels are observed in a large proportion of spines, which shows strong correlation with the adjacent dendrites. This suggests that local calcium homeostasis compartmentalization has been lost and calcium leaks out of the spine into the dendrite, preventing independent calcium signaling of spines and dendrites (Green & LaFerla 2008). $A\beta_{42}$ may disrupt intraneuronal calcium levels and regulation by inducing oxidative stress (increased levels of 4-HNE) which impairs membrane calcium pumps and enhances calcium influx through voltage-dependent channels and ionotropic glutamate receptors (Mattson & Chan 2003). It has also been shown that addition of Aβ leads to abnormal functioning of the $Na^+/K^+$-ATPase (Colom et al 1998), which in turn could lead to increased levels of intracellular $Ca^{2+}$ via increased levels of intracellular $Na^+$ which triggers membrane depolarization (Good & Murphy 1996). Muscarinic acetylcholine receptors (mAChRs) are also targeted by Aβ, leading to inositol triphosphate ($IP_3$) and subsequent $Ca^{2+}$ release from intracellular calcium stores via activation of G-proteins and phospholipase C (PLC) (Kelly et al 1996). A schematic summary of different membrane proteins involved in Aβ induced calcium dyshomeostasis is given in figure 5.

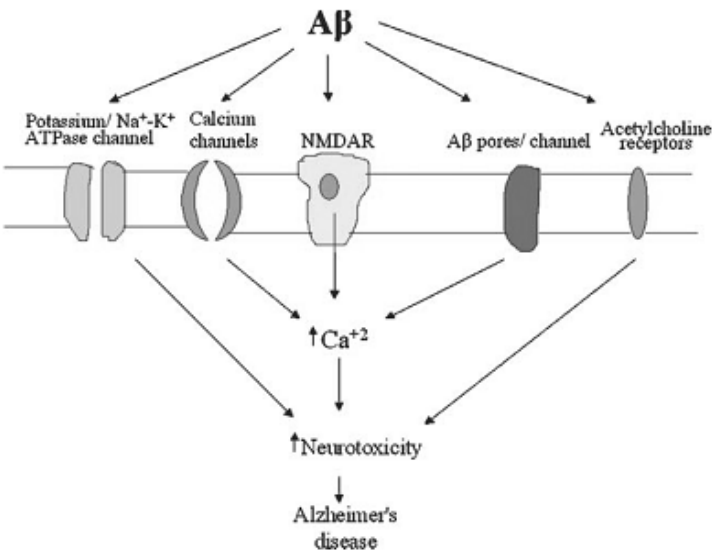


Figure 5. Membrane protein affected by Aβ. Figure adopted from Sultana & Butterfield 2008.(Sultana & Butterfield 2008)

Excessive and sustained calcium levels also induce free-radical production by altering mitochondrial oxidative phosphorylation and activating oxygenases, which makes it likely that perturbed calcium homeostasis and free-radicals are components of a self-amplifying cascade (Bezprozvanny & Mattson 2008). Further, it has been described that in the presence of Aβ peptides there are ion conducting channels formed in the membrane, which could possibly be described to the fact that Aβ oligomers share structural and functional homology with pore-forming bacterial toxins and perforin (Yoshiike et al 2007). Neurons with exposure of phosphatidylserine, which is indicative of apoptotic or energy deprived cells, show enhanced Aβ binding (Lee et al 2002). Therefore, it is possible that age-related mitochondrial impairments facilitate Aβ-mediated pore formation and calcium influx. In addition, cell-surface receptors coupled to calcium influx are activated and calcium release from the endoplasmic reticulum (ER) is enhanced (Mattson & Chan 2003). The activation of calpains and caspases and the dysregulation of calcium homeostasis were shown to be involved in impaired neuronal survival, cell proliferation and differentiation induced by Aβ (Haughey et al 2002). The main site of calcium dysregulation seems to be the synapse, where Aβ impairs plasma membrane $ca^{2+}$ ATPase in exposed synaptosomes (Mark et al 1995). Amyloidogenic processing of APP can impair neuronal calcium homeostasis by decreasing the production sAPPα, a soluble secreted form that activates $K^{+}$-channels (Furukawa et al 1996).

## Aβ mediated endoplasmic reticulum and mitochondrial dysfunction

The ER serves as a dynamic store for calcium, with a high calcium concentration being maintained within its lumen. Maintenance of the steep concentration gradient between the cytoplasm and the ER lumen is regulated by calcium uptake through the sarcoplasmic/endoplasmic $Ca^{2+}$-ATPase (SERCA) pathway and is released through ryanodine receptors and $IP_3$ receptors. Protein synthesis, folding, assembly and transport are all calcium dependent processes and are thus influenced by impairments in ER calcium homeostasis. Aβ treatment evokes calcium leakage from the ER via $IP_3$ and ryanodine receptors, which is involved in the activation of apoptotic cell death in cells stressed with Aβ peptides (Ferreiro et al 2004). Very recently, it was found that soluble, oligomeric species of Aβ disrupted the anchoring of the ER to microtubules and thereby decreased the stability of the ER and the microtubules and promoted ER collapse and enhanced lysosomal degradation (Lai et al 2009).

In AD brain, energy metabolism is severely compromised. For example, pyruvate dehydrogenase (PDH) and α-ketoglutarate dehydrogenase (KGDH) activity is decreased in the frontal, parietal and temporal cortex of AD brain, which does not correlate with expression of these enzymes, suggesting that inhibition accounts for the decreased activity (Gibson et al 1998). Depressed cytochrome c oxidase (COX) activity has also been found in brain homogenates form AD patients (Kish et al 1999), which is probably the result of structural changes in the binding site, because kinetic behavior of the enzyme is altered (Parker & Parks 1995). In brain imaging studies it has been demonstrated that there are deficits in glucose consumption which seems to occur before the onset of clinical symptoms (Blass 2001), and mitochondrial degeneration occurs before NFTs are evident (Hirai et al 2001). This indicates that mitochondrial failure could be an early event in the pathogenesis of AD. Aβ peptides generated directly in the mitochondria may be responsible for the mitochondrial dysfunction that occurs in AD. The mechanism seems to involve enhanced ROS production (Arias et al 2002), to which several respiratory chain enzyme complexes are particularly vulnerable (Casley et al 2002). Moreover, Aβ causes damage to the respiratory chain and leads to opening of the mitochondrial permeability transition pore (Moreira et al 2002), which promotes cytochrome c release and triggers apoptotic cell death (Kim et al 2002). Aβ progressively accumulates in the mitochondrial matrix, which is associated with decreased activity of enzymatic respiratory chain complexes III (succinate-cytochrome c reductase) and IV (COX), as well as decreased oxygen consumption (Mucke et al 2000). Since COX directly interacts with molecular oxygen, loss of COX could lead to increased superoxide side production. Another possible target for mitochondrial Aβ-mediated toxicity is a short-chain alcohol dehydrogenase which specifically binds to Aβ, Aβ binding alcohol dehydrogenase (ABAD) (Lustbader et al 2004), which expression is increased in AD brain, enhancing Aβ induced cell stress and cytotoxicity (Yan et al 1997). Antagonizing ABAD/Aβ interaction protects against Aβ induced neuronal and mitochondrial toxicity by decreasing cytochrome c release, DNA fragmentation, lactate dehydrogenase (LDH) release and

generation of ROS, which is consistent with data from APP/ABAD transgenic mice. These mice exhibited mitochondrial dysfunction and impaired behavioral and synaptic function (Chen & Yan 2007). Figure 6 summarizes some observed effects of Aβ/ABAD interaction in the mitochondria.

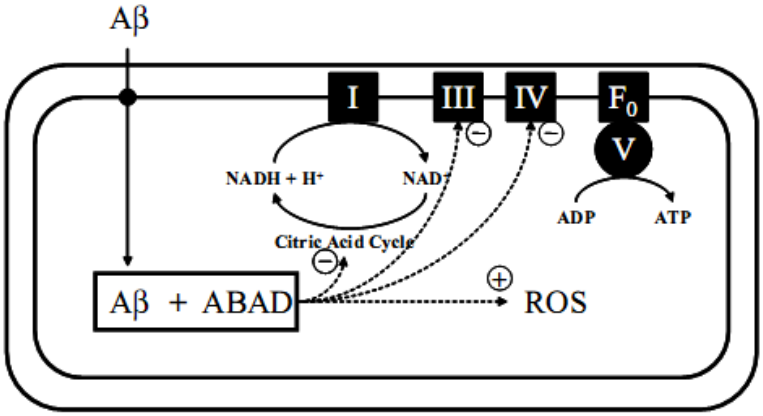


Figure 6. Schematic diagram of the consequences of interaction between Aβ and ABAD. Figure adopted from Chen and Yan 2007.

Mitochondria and the ER have close physical contact and for normal cell functioning, it is essential that both organelles interact. In the pathogenesis of neuronal cell death, apoptotic cross-talk between the mitochondria and the ER has been identified (Hacki et al 2000), which suggests that ER stress might be an upstream mediator of mitochondrial dysfunction and neuronal cell death as seen in AD (Katayama et al 2004). In mice neurons stressed with Aβ peptides, the knock-out of the ER resident caspase-12 made the cells resistant to cell death caused by Aβ (Nakagawa et al 2000), similar results are found with the human analog caspase-4 (Hitomi et al 2004).

## Aβ induced cholinergic dysfunction

It is thought that, in the pathogenesis of AD, dysfunction and loss of basal forebrain cholinergic neurons and their cortical projections are among the earliest pathological events. In addition to the large neuronal loss in these brain regions, the evidence pointing to cholinergic impairments come from studies that report a decline in the activity of choline acetyltransferase (ChAT) and acetylcholine esterase (AChE), acetylcholine (ACh) release and the levels of nicotinic and muscarinic receptors in AD brain. Considering the role of Aβ in cholinergic dysfunction, it has been stated that ACh release and synthesis are depressed and ACh degradation is affected in the presence of Aβ peptides (Auld et al 2002). It was found that Aβ inhibits the fast axonal transport of vesicular ACh transporter (VAChT), which supports the idea that AD results of failures in axonal transport (Kasa et al 2000). Although AChE levels are reduced in AD brain, its activity is increased around plaques and in NFT bearing neurons (Talesa 2001). The increase in activity of this enzyme is likely to be due to an indirect effect of Aβ, mediated via oxidative stress (Melo et al 2003), via voltage dependent calcium channels or via nAChRs of the α7 subtype (Fodero et al 2004). $A\beta_{42}$ was far more potent then $A\beta_{40}$ in increasing AChE enzymatic activity. Aβ has been shown to bind to α7-nAchRs, affecting its nicotinic currents (Pettit et al 2001) and Erk/MAPK signaling, eventually leading to the down regulation of Erk/MAPK and decreased phosphorylation of CREB protein (Dineley et al 2001). nAChRs are ligand-gated ion channels of which α7-nAchRs is $ca^{2+}$ permeable. Normal nicotine binding prevents activation of NFκB and c-Myc by inhibiting the activation of MAPKs, as a result, the activity of iNOS and consequent production of NO are down-regulated (Liu et al 2007). In AD brain iNOS and NO are upregulated. Another study showed the involvement of a different signaling pathway associated with α7-nAchRs, Aβ binding prevents normal nicotine binding, especially the binding of $A\beta_{42}$ to α7-nAchRs is exceptionally high as compared to $A\beta_{40}$ (Wang et al 2000). Recently, a novel nicotinic ACh receptor subtype has been identified, the heteromeric α7β2-nAchR, and this subtype is highly sensitive to low concentrations of oligomeric $A\beta_{42}$ but not monomeric or fibrillar forms (Liu et al 2009). It has been suggested that Aβ-α7-nAchR binding facilitates the internalization and accumulation of Aβ in the neuron. Cells that express relatively high levels of α7-nAchRs show substantial Aβ accumulation, which is prevented by the selective α7-nAchR antagonist α-bungarotoxin (Nagele et al 2002). mAChR signaling pathways are also impaired by Aβ. In APP/PS1 double transgenic mice, the density of mAChRs was lowered, which undergoes an age related decline that is not solely attributable to mAChR depletion alone, but rather to a malfunction in mAChR-G-protein coupling (Machova et al 2008). Activation of mAChRs could be neuroprotective via mitogenic Wnt signal transduction pathways (Wnt signaling is described under '*The role of Wnt signaling'*) (Farias et al 2004). Furthermore, it has also been found that AChE promotes Aβ aggregation, possibly through Aβ-AChE interaction by a hydrophobic environment close to the peripheral anionic binding site of the enzyme, thus promoting fibril formation (Inestrosa et al 1996). When AChE becomes associated with amyloid fibrils, some of its characteristics, like sensitivity to low pH,

change. Therefore, AChE might play an important role in neurotoxicity induced by Aβ. This notion is supported by the observation that Aβ-AChE complexes are more toxic than amyloid fibrils alone (Alvarez et al 1998).

However, the relationship between Aβ and the cholinergic system is not unidirectional. There is a considerable amount of evidence that cholinergic dysfunction influences APP metabolism and consequent Aβ production. For example, it has been shown that stimulation of the M1 and M3 muscarinic receptor subtypes increased the release of APP through activation of PLC/protein kinase C (PKC) cascade (Nitsch et al 1992). BACE expression was also increased by activation of these receptor subtypes (Zuchner et al 2004). Together this results in increased Aβ secretion.

## Aβ induced apoptosis

Several mechanisms have been proposed that mediate Aβ-induced neuronal apoptosis. As described above, neuronal Aβ exposure results in an increase in cellular calcium concentration, which triggers the activation of calpains. Calpains are calcium-dependent neutral proteases, which becomes activated following alterations in intracellular calcium homeostasis. This leads in run to DNA fragmentation by cleavage of poly-ADP-ribose polymerase, a DNA repair enzyme (Boland & Campbell 2003). The rise in cellular calcium levels, induced by Aβ treatment, leads to the expression of neuronal death protein 5 (DP5 or Hrk), which in turn binds to B-cell lymphoma-extra large (Bcl-xl, a member of the Bcl2 family), an anti-apoptotic mitochondrial transmembrane protein, thereby impairing the survival-promoting activities of Bcl-xl (Imaizumi et al 1999). In cortical neurons exposed to Aβ, it has been found that activation of JNK is required for phosphorylation of the c-Jun transcription factor, which in turn stimulates the transcription of the death inducer Fas ligand. Consequently, the binding of Fas ligand to its receptor Fas induces a cascade of events that lead to caspase activation and ultimately cell death (Morishima et al 2001). Aβ binds to $p75^{NTR}$ and induces cell death through p75-like apoptosis inducing death domain (PLAIDD), inhibitory G-protein, JNK, reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and caspase-3 and caspase-9 (Tsukamoto et al 2003). Aβ exposure to cerebral endothelial cells induced translocation of second-mitochondria-derived activator of caspase (Smac), an apoptosis regulator, from the mitochondrial intramembranous compartment to the cytosol which binds to X chromosome-linked inhibitor-of-apoptosis protein (XIAP). In addition, Aβ treatment also led to activation of the transcription factor AP-1 and subsequent Bim expression, a pro-apoptotic protein. Together, these events lead to cerebral endothelial cell death (Yin et al 2002). In transgenic mice and AD brain it was demonstrated that Aβ accumulation triggers caspase activation which in turn lead to caspase mediated cleavage of tau, which converts tau into an effector of apoptosis (Fasulo et al 2000).

## Aβ mediated neuroinflammation

The notion that inflammatory processes are involved in the pathogenesis of AD is strongly supported by epidemiological studies, indicating that chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) reduces the risk of developing AD (Stewart et al 1997). The neuroinflammatory process in AD involves astrocytes, microglia, the complement system and to a lesser extent neurons (Akiyama et al 2000). Senile plaques are known to be associated with activated microglia and reactive astrocytes, with the extend of activation directly dependent on amyloid load (McGeer & McGeer 1995). However, the glial activating possibly depends on protofibrillar Aβ species because it was found that glial activation precedes plaque burden (Heneka et al 2005). Microglial interaction with these amyloid deposits triggers the phenotypic activation and, as a consequence, a number of pro-inflammatory immune receptors and cell surface proteins are overexpressed, such as the leukocyte antigen CD45, complement receptors such as CR3 and CR4, Lymphocyte function-associated antigen 1 (LFA-1), major histocompatibility complex II (MHC II) surface antigens and the type 1, 2 and 3 Fcγ immunoglobulin receptors. Moreover, the acute phase proteins amyloid P and C-reactive protein and the protease inhibitors α1-antichymotrypsin and α1-antitrypsin are elevated (Tuppo & Arias 2005), all indicating inflammatory upregulation. $A\beta_{42}$ strongly activates DNA binding to NFκB, which modulated inflammatory gene expression, via ROS intermediates in neuronal cells (Kaltschmidt et al 1997). Astrocytes migrate to the sites of Aβ deposition in response to monocyte chemotactic protein-1 (MCP-1), which levels are increased in AD brain (Galimberti et al 2006).

There is also a large body of evidence reporting that fibrillar Aβ peptides induce the synthesis of pro-inflammatory factors interleukin-1 (IL-1), IL-6 and tumor necrosis factor-α (TNF-α)(cytokines) and macrophage inflammatory protein-1 and IL-8 (chemokines) and release from microglia via ERK/MAPK pathways (Yates et al 2000). These factors stimulate astrocytes to release cytokines, chemokines and acute phase proteins, which in turn activates the microglia to further increase the inflammatory response. Moreover, IL-1 and IL-6 have been shown to activate MAPK-p38 signaling and cdk5/p35 (Quintanilla et al 2004), which is involved in tau hyperphosphorylation (see '*tau hypothesis*'), linking inflammation and tau pathology. Expression of IL-1 is also involved in iNOS activation in hippocampal neurons (Serou et al 1999), which might account for some part of the increased oxidative stress observed in AD. The increased levels of pro-inflammatory cytokines in AD brain can affect Aβ formation by raising the susceptibility for aggregation and deposition (Guo et al 2002), by upregulating BACE activity and transcription (Sastre et al 2003) and by increasing APP synthesis and half-life (Amara et al 1999; Rogers et al 1999).

TNF-α, which serum levels are increased in AD (Alvarez et al 1996), is produced by microglia in response to Aβ peptides. Its neurotoxic effect involves induction of inflammatory tissue damage and activation of its receptor, TNF-Receptor 1 (Li et al 2004). TNF-related apoptosis inducing ligand (TRAIL) is specifically expressed in AD brain, and completely absent in healthy

brain. TRAIL can be induced by Aβ in neurons and in astrocytes stimulated with cytokines and neutralization of TRAIL protects neurons form Aβ induced neurotoxicity (Cantarella et al 2003).

The interaction of Aβ fibrils with microglia seem to involve a cell surface receptor complex including the B-class scavenger receptor CD36, $\alpha_6\beta_1$ integrin and CD47 (Bamberger et al 2003). Activation of this receptor complex induces activation of intracellular tyrosine kinases. Further, the following microglial cell-surface receptors which bind to Aβ have been identified: scavenger receptor A (SRA) (El Khoury et al 1996), RAGE (Yan et al 1996) and the serpin enzyme complex (SEC) receptor (Boland et al 1996). SRA acts principally to mediate cellular adhesion to Aβ fibrils. SEC binds only to soluble, non-aggregated Aβ, after which Aβ is internalized and degraded.

Complement factors are associated with plaques and it is known that the classical complement pathway is activated by Aβ fibrils (Fan & Tenner 2004), acute phase proteins amyloid P and C-reactive protein (Elward & Gasque 2003) and tau protein (Shen et al 2001). Activation of complement cascade pathways can lead to enhanced forming of the membrane attack complex (MAC), which induces cell death in nearby cells by compromising membrane integrity (Elward & Gasque 2003).

As described earlier, treating neuronal cells with Aβ, increases NGF secretion. Increased NGF in AD brain is not found only in neuronal cells, but also in astrocytes and microglia (Siegel & Chauhan 2000). In addition, it has been found that Aβ is a potent stimulator of microglial NGF synthesis (Heese et al 1998). Moreover, Aβ incubated astrocytes, show upregulated NGF expression and secretion, and when placed in culture with hippocampal neuronal cells, the hippocampal cells show increased tau phosphorylation and decreased survival (Saez et al 2006). This together indicates a role for NGF in Aβ induced toxicity.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear hormone receptors and transcription factors which are strongly implicated in the control of inflammatory responses (Cullingford et al 1998). There is also a consistent amount of evidence indicating that PPAR ligands are able to modulate brain inflammatory responses in order to reduce neuronal damage in neurodegenerative diseases like AD (Sastre et al 2006). Ligands for the PPAR subtype, PPARγ, have been shown to inhibit activation of microglial cells induced by Aβ. PPARγ ligands also reduce iNOS, cyclooxygenase-2 (COX-2, not to be confused with cytochrome c oxidase, which is also abbreviated as COX) and TNF-α expression caused by Aβ stimulation of cell cultures (Landreth & Heneka 2001).

NSAID treatment did not only reduce the risk for developing AD, it also preferentially decreases $A\beta_{42}$ levels (Gasparini et al 2004). An aggregation study pointed out that several NSAIDs dose-dependently inhibited formation of fibrillar from soluble $A\beta_{42}$ and destabilizes the already formed fibrillar species (Hirohata et al 2005). It was also found that only those NSAIDs, who preferentially block Rho, are able to reduce the levels of $A\beta_{42}$ (Zhou et al 2003).

However, clinical studies have indicated that NSAIDs cannot improve the pathogenesis of AD in patients although NSAIDs seem to be efficient in lowering the risk of developing AD (Townsend & Pratico 2005).

It is likely that in advanced stages of AD, neuroinflammation is not pivotal for the clinical symptoms. Franceshci and colleagues introduced the term 'inflammaging,' to characterize the paradigm that ageing was accompanied by chronic upregulation of certain inflammatory responses (Franceschi et al 2007). Inflammaging differs from inflammation in that it is low-grade, controlled, asymptomatic, chronic and systematic. The end result is a cycle of chronic and systemic pro-inflammatory responses where both tissue damaging and healing mechanisms operate simultaneously. The over-active immunity which is characteristic for inflammaging remains sub-clinical in most elderly, however, a portion of individuals may shift from a state of sub-clinical inflammaging to an age-associated disease, like AD, likely as a result of chronic increased ROS formation, which might precede Aβ production, as described earlier (Nunomura et al 2001).

**Conclusion**

In general, soluble levels of Aβ in brain are considered the best correlate of neuronal degeneration and cognitive decline in AD by now. The extracellular plaques, which used to be considered the main cause of AD pathology, are increasingly recognized as tombstones. Many hypotheses have been tested over the past years, of which many are approved after extensive research. However, it still remains to be established how Aβ exerts its neurotoxic properties. By now, synaptic dysfunction triggered by Aβ species is considered an early event in the pathogenesis of AD. The main pathogenic events that may contribute to synaptic dysfunction include oxidative stress and oxidative stress related signaling, dysregulation of calcium homeostasis, impairments in the mitochondria and the ER and cholinergic dysfunction, which all have been shown to be influenced by Aβ species. Further, inflammatory signals in the brain and inhibition of growth signaling are potential candidates mediating the neurotoxicity induced by Aβ. The responsible Aβ species has not been identified yet and it is unlikely it will be identified in the near future, considering the results from experiments with many different species of Aβ. It is reasonable to suggest that AD is not the consequence of a single Aβ species but that different species could be responsible for the pathologic events. It is also quite possible that different species of Aβ have different roles at different locations, such as the cytosol, the mitochondria or the CSF. Moreover, different cell types (e.g. microglia, neurons, etc.) might behave differently when exposed to Aβ species. According to me, it seems really likely Aβ is not the one and only cause of AD pathology, but many systems and mechanisms in the brain are affected. Therefore, the suggested view from Pimplikar et al. 2009, seems really compelling, placing Aβ at the same level as other pathologic hallmarks. By acting in concert this eventually leads to the progressive cognitive decline associated with AD. Failure in one these components (Aβ, NFT, inflammation, etc.), or failure upstream of them, could trigger further failure in all of these components resulting in full AD pathology. It should be noted, that at some time point in the progression of AD, Aβ might be responsible for all of the further pathologic events observed in AD. According to this view, for a therapy to be successful, it should involve all of the affected components in AD, because treatment focusing at Aβ only will fail because the other affected components still trigger the misfolding of Aβ, restoring the pre-therapeutic situation.

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