As Bad as its Reputation?
The Physiological Function of Amyloid Precursor Protein and Amyloid-β

Bachelor Thesis

Neele Meyer
s1610848
University of Groningen, The Netherlands

Groningen March/April 2009

Supervised by: Ivica Granic
Abstract

Alzheimer’s Disease is a common neurodegenerative disorder in which the Aβ protein is thought to play an important causative role. For a long time it was supposed that Aβ only has toxic characteristics and is a waste product of APP (Amyloid Precursor Protein) cleavage, however more and more studies find evidence for possible physiological functions of Aβ. APP seems to play an important role in preventing cells from excitotoxicity in a negative feedback manner. Additionally, if applied in physiological amounts, Aβ improves memory formation. Moreover Aβ is part of the lipid metabolim in which it reduces cholesterol levels. APP is important for cell movement in the developing and adult brain, for example by accelerating wound healing. The AICD (APP intracellular domain) is involved in gene transcription and also seems to be a “cell-death mediator”. These findings call for a reassessment of today’s drugs and drug targets against Alzheimer’s Disease, which mostly focus on inhibiting Aβ production or a fast Aβ removal and could thus have a damaging effect for the patient.
# Table of Content

Abstract .................................................................................................................................................. 2

Table of Content.................................................................................................................................... 3

Introduction ........................................................................................................................................... 4

1. Physiology of APP processing and production of Aβ ......................................................................... 4
   1.1 The two pathways: Amyloidogenic and non-amyloidogenic ....................................................... 5

2. Physiological role of APP .................................................................................................................. 6
   2.1 Formation of Neuromuscular Synapses and in cortical development ........................................ 7
   2.2 Role in cell migration and motility ............................................................................................... 7
   2.3 Enhancing postsynaptic NMDAR expression ............................................................................... 9

3. Physiological roles of Aβ .................................................................................................................. 10
   3.1 Synaptic plasticity and negative feedback in neuronal activity ................................................. 10
   3.2 Lipid metabolism ....................................................................................................................... 14

4. Physiological roles of AICD ............................................................................................................. 15
   4.1 Involvement in gene transcription ............................................................................................. 15

Summary and Conclusions .................................................................................................................. 18

References ........................................................................................................................................... 19
Introduction

Alzheimer’s disease (AD) is the most common progressive neurodegenerative disease. The Amyloid-β protein (Aβ) is believed to play an important role in AD, forming toxic oligomers that lead to cell death resulting eventually in cognitive impairments. Aβ is formed by cleavage of the Amyloid Precursor Protein (APP), a type I-transmembrane protein. Mostly Aβ<sub>1-42</sub> is known to be toxic probably because it aggregates faster than other Aβ peptides. Also there is more Aβ<sub>1-42</sub> present in Alzheimer brain than in healthy brains whereas there is far more Aβ<sub>1-40</sub> in healthy brains (Small et al 2006). For a long time it was supposed that Aβ only has toxic characteristics and is a waste product of APP cleavage. However, this view is challenged as more and more studies find evidence for possible physiological, not pathological, functions of Aβ such as participation in synaptic function, memory formation and neuronal survival. Additionally, there is also a lot of Aβ found in healthy subjects, both in the brain and the rest of the body, what hints at one or more possible physiological roles (reviewed by Bishop et al 2004).

As many medications for AD now focus on the inhibition of Aβ production or reduction of Aβ load, such as γ- secretase inhibitors or vaccination against Aβ, it is also important to know about possible physiological functions (reviewed by Hüll et al 2006). If Aβ has important physiological functions these drugs could have devastating effects on the patients. This was the reason for me to focus my thesis on the possible physiological roles of APP, Aβ and other APP cleavage products.

1. Physiology of APP processing and production of Aβ

The Amyloid Precursor Protein (APP) is a type –I transmembrane protein that belongs to a protein family that includes APL-1 in C. elegans, APPL in Drosophila and Amyloid Precursor Protein like Protein 1 and 2 (APLP1 and APLP2) in mammals (reviewed by Zhang 2007). These proteins share several conserved domains such as E1 and E2 in the extracellular part and the AICD (APP intracellular domain), however the Aβ domain is only found in APP (reviewed by Zheng 2006). APP consists of a large extracellular N-terminal (aa 28-128) and a short intracellular (cytoplasmic) C-terminal (Reinhard 2005).

The gene encoding for APP lies on the long arm of chromosome 21 in humans. Alternative splicing results in the production of several isoforms, ranging from 365 to 770 amino acid residues. The major Aβ peptide encoding isoforms are APP695, APP751, APP770, consisting of 695, 751 and 770 amino acids respectively. Whereas APP 751 and APP 770 are mostly found in the brain but also in the rest of the body, APP 695 is predominantly found in
neurons. However, it is not known yet why APP 695 shows tissue specificity. Moreover APP 751 and APP 770 contain a Kunitz Protease Inhibitor (KPI) domain (Zheng 2006).

Full length APP is synthesized in the endoplasmatic reticulum (ER) and transported through the Golgi to the trans-Golgi-network (TGN) which is the major site of APP residence in neurons at steady state. It is transported to the cell surface in TGN derived vesicles (Zhang 2007).

1.1 The two pathways: Amyloidogenic and non-amyloidogenic

APP can be proteolytically processed in 2 pathways, the amyloidogenic pathway in which Aβ is generated and the non-amyloidogenic pathway (Zhang 2007).

In the non-amyloidogenic pathway APP is cleaved at the cell surface by α-secretase, a zinc-metalloproteinase, probably consisting of members of the ADAM (a disintegrin and metalloproteinase) family. By cleavage the soluble ectodomain of APP is released (sAPPα) and a C-terminal stub (CTFα, C-terminal APP fragment α, or C83) are formed. Subsequently, γ-secretase cleaves CTFα which leads to the release of AICD (APP intracellular domain) and the P3 fragment. The γ-secretase is a complex protein consisting of at least four subunits; presenilin (PS), nicastrin, APH-1 and presenilin enhancer-2 (PEN-2). Interestingly, α-secretase cleaves within the Aβ-domain so that no Aβ is formed in this pathway (Zhang 2007).

If APP is not cleaved by α-secretase it can be reinternalized to an endosomal/lysosomal pathway. Here it can enter the amyloidogenic pathway where it is cleaved by β-secretase, a transmembrane protease, BACE1 (β-site of APP cleaving enzyme) (reviewed by Small 2006). Again, a soluble ectodomain is secreted (sAPPβ) and a C-terminal stub (CTFβ or C99) is generated.

Subsequently γ-secretase cleaves CTFβ which leads to the release of AICD and Aβ. The γ-secretase cleaving site is variable, so Aβ of different length are formed, ranging from 39 – 43 amino acids in length. In healthy subjects mostly Aβ1-40 (consisting of 40 amino acids) is found, in AD however a shift is noticed leading to a Aβ1-40 – Aβ1-42 ratio of about 50% each (discussed in Plant et al 2003). Aβ can be degraded by enzymes such as neprilysin, insulin degrading enzyme and an endothelin enzyme (reviewed by Pearson et al 2006).
2. Physiological role of APP

The quest to find and understand the physiological function of APP is hampered/impeded by the fact that APP, APLP1 and 2 have partially redundant functions making reverse genetic approaches complicated (Anliker et al 2006). APP knock-out (APP ko) mice are viable and fertile and showed a specific subtle phenotype: retarded somatic outgrowth, reduced grip strength, increased copper levels in the cerebral cortex, impaired LTP and behavioral deficits (reduced locomotor activity, reduced exploratory behavior and impaired spatial learning). In vitro analysis showed a slightly reduced survival rate and impaired neurite outgrowth in hippocampal neurons, whereas cortical neurons didn’t show such changes (Anliker et al 2006).

APLP1 deficient mice showed a somatic growth deficit whereas APLP2 ko mice appeared normal. A combined knock-out of APP/APLP2 or APLP1/APLP2 resulted in perinatal lethality,
they died short after birth. Very few APP/APLP2 ko mice survived into adulthood and showed a more severe/enhanced phenotype of the APP ko mice (Anliker et al 2006).

APP/APLP1 ko was viable and fertile and showed the same phenotype as APP ko. These results demonstrate functional redundancy between the APP family members and shows that APP (and its family members) are crucial for early postnatal development (Anliker et al 2006).

2.1 Formation of Neuromuscular Synapses and in cortical development

APP and APLP2 seem to play an important role in the development of neuromuscular junctions (NMJs). NMJs of APP/APLP2 knock-out mice are characterized by aberrant presynaptic structures consisting of excessive nerve terminal sprouting and an impaired apposition of presynaptic marker protein with postsynaptic ACh receptors. Probably there is also a reduction in synaptic vesicle density because neurotransmission is severely impaired (highly reduced frequency of miniature endplate potentials). These changes were only found at the NMJs, not in the brainstem (Anliker et al 2006).

A triple ko also survives through embryonic development but dies shortly after birth. These animals show a focal cortical dysplasia which resembles human type II lissencephaly which is an cranial abnormality. One reason might be a defect in neuronal migration during gestation. The total number of Cajal-Retzius cells was reduced along the entire cortex in triple-knock-out animals (Priller et al 2006).

2.2 Role in cell migration and motility

It was further suggested by Sabo and colleagues that APP might play a role in cell motility. This is probably achieved by forming a macromolecule by binding of FE65 and Mena. It was shown that APP and FE65 selectively colocalize with Mena in adhesion sites of mobile membranes (focal complexes) at the base of protruding lamellipodia, but not in static membranes (focal adhesions). The protein Mena (mammalian Ena) is the mammalian homologue of Drosophila Enabled (Ena) and is required for normal neural development and regulates the actin cytoskeleton by interacting with profilin (Gertler et al 1990). This links APP and FE65 indirectly to the lamellipodial cytoskeleton (Sabo et al 2001). Probably FE65 interacts with APP through its PID domain and with Mena trough its WW domain. Mena modulates actin polymerization by interacting with profilin (Sabo et al 2001). See figure 2.

Cell adhesion:
As the focal complexes and adhesions consist amongst others of integrin, a family of cell adhesion receptors it might also play a role in regulating cell adhesion. It was found that the
APP-FE65- Mena complex colocalizes with β-integrin (a subunit of integrin) in lamellipodia. The complex might help destabilizing adhesion sites making it more prone to cell movement. (Sabo et al 2001)

Cell extension:
The APP-FE65-Mena complex localizes in protruding lamellipodia indicating a role in cell movement. An indicator for the rate of cell migration is wound-healing. To test this, cells were injured and wound-healing was observed. Indeed APP and FE65 accumulated in wound –induced lamellipodia, thus possibly regulating cell motility. This is further supported by the finding that overexpression of APP and FE65 accelerate wound healing. Furthermore, an overexpression of APP and FE65 increased the rate at which the wound edge cells traveled as compared to control cells. This indicates that APP and FE65 are involved in regulation of cell movement, however whether this is achieved by changing actin dynamics or altering adhesion is not known yet (Sabo et al 2001). This is also in line with the finding that more APP is produced as a reaction to neural damage and during brain development (reviewed by Koistinaho et al. 2007 and Wasling et al 2009).

It is of importance to notice that the FE65 and profilin binding sites on Mena overlap. This is why Sabo suggested that the APP-FE65 complex negatively regulates the binding of Mena to profilin. Lamellipodial Mena was shown to decrease cell motility in fibroblasts, probably by binding to Robo, a transmembrane receptor that mediates repulsion of axons. It is suggested that normally Mena binds to Robo, negatively regulating cell motility. In response to a certain signal, for example overexpression of APP or FE65, however, Mena is recruited to the APP-FE65 complex (discussed in Sabo 2001).

Figure 2: A schematic depiction of possible components of the macromolecule involved in the regulation of membrane motility. FE65 binds APP, Mena and integrin β. Interaction of Mena with profilin (not shown) might modulate actin polymerization. Integrins are indirectly linked to actin via different integrin and actin binding proteins, eg. α-actinin. (from Sabo et al 2001).

Furthermore it was recently found by Young-Pearse et al. that sAPP regulates the function of full-length APP by interacting with integrin-β. As mentioned above APP may interact with integrins leading to cell motility and thus could lead to neurite outgrowth. APP
and integrins colocalize in developing neurons in both growth cones (filopodia and lamellipodia) and along axons. It seems that APP knock-out cultures have longer axons than wild-type cultures, indicating that APP inhibits neurite outgrowth (Young-Pearse et al 2008). This seems to be in conflict with the results from Sabo et al. However, it was shown that the addition of sAPPα reversed the inhibiting effect. Moreover, this effect could not be observed in APP knock-out cultures what may denote that APP and sAPPα might bind to the same factors, most probably Integrin-β. sAPPα could thus compete with APP and inhibit its neurite-outgrowth regulating activity (Young-Pearse et al 2008).

It might be that the same phenomenon occurred in the experiments Sabo did, as they didn’t look for effects of APP fragments. However, this seems unlikely because Sabo did coimmunoprecipitation that indicated that APP and not one of the fragments bind to integrin. Another possibility might be that the effect of APP is different depending on the type of neuronal tissue, the timing and the substrate upon which the neurons are plated (Young-Pearse et al 2008).

In another study, Young-Pearse et al showed by in utero electroporation of shRNA constructs into the developing cortex, a method to acutely knock down APP in rodents, that APP is required for neuronal migration in the developing brain, more specifically for migration of neuronal precursor cells into the cortical plate. In APP deficient rats the neuronal precursors were able to migrate out of the ventricular zone and into the immediate zone but were not able to enter the cortical plate as they accumulated just below the plate. On the other hand, overexpression of APP led to a faster migration into the cortical plate. This was explained by suggesting that the cortical plate expresses extracellular factors that are different from the intermediate zone and that the binding of the APP ectodomain to these factors influences the differential adhesion between intermediate zone and cortical plate and this in turn mediates migration past this boundary (Young-Pearse et al 2007).

Additionally, they showed that the whole APP is necessary for proper migration. Application of sAPPα/β or AICD to APP reduced neurons did not restore migration. Thus it can be assumed that full-length APP and not one of its fragments plays an important role in brain development (Young-Pearse et al 2007).

From the studies discussed above it can be concluded that APP might play an important role cell migration in the developing brain as well as in the adult brain.

2.3 Enhancing postsynaptic NMDAR expression

One recent finding is that APP affects postsynaptic composition and activity. Hoe et al found full-length APP in postsynaptic densities and showed that it increases NMDA receptor
current density and thus enhances receptor function (Hoe et al 2009). Furthermore, it was shown that APP increases the surface expression of the NMDA receptor subunit NR2B. This subunit is known to play a role in synaptic plasticity and even though its precise role is not determined yet, it is most likely to be involved in LTP (Long Term Potentiation) and LTD (Long Term Depression). The NR2B contacts a distinct set of signalling proteins and has slow deactivation kinetics favouring greater Ca$^{2+}$ influx which leads to higher synaptic activity in response to neuronal damage and during learning. Moreover, APP decreases NR2B internalization which, together with the increased surface expression leads to increased synaptic activity (Hoe et al 2009).

APP and Aβ are both produced in response to neuronal damage (reviewed by Koistinaho 2007). While APP increases NMDA receptor activity, it is thought to be reduced by Aβ (described later in part 3.1). This counteracting effect could be a physiological “brake-mechanism” to prevent excitotoxic effects if synaptic levels become excessive. Neural damage increases APP expression which leads to more APP processing and thus more Aβ production. In turn Aβ reduces APP-induced synaptic activity (Hoe et al. 2009).

3. Physiological roles of Aβ

3.1 Synaptic plasticity and negative feedback in neuronal activity

The most important glutamatergic receptors are AMPA and NMDA receptors. They have an excitatory effect and can undergo changes in their strength in response to activity, which is a prerequisite for synaptic plasticity. Synaptic plasticity, characterized by long term potentiation (LTP) and long term depression (LTD), is believed to be the cellular correlate of memory regulation (reviewed by Parameshwaran 2008). This is mostly achieved by NMDA receptor-dependent LTP and LTD (Bear pp 778-782).

By a coincident presynaptic glutamate release and postsynaptic depolarization Ca$^{2+}$ enters through the NMDA receptor. The elevated level of Ca$^{2+}$ activates a cascade that includes several kinases, among others CaMKII and PKC. These lead to an increase in efficiency of AMPA receptors or the insertion of new AMPA receptors into the membrane. This is called LTP. For LTD a lower rise in Ca$^{2+}$ is required and leads eventually to a removal of AMPA receptors from the membrane (Bear pp 778-782).

Kamenetz et al proposed a possible role of Aβ in negative feedback signaling in neuronal activity. They found that the application of pharmacological agents that either decrease or increase neural activity onto slices of APP$_{SWE}$ (human APP with Swedish mutation) mice brains resulted in a reduction or elevation of Aβ respectively. Interestingly
this is true for both Aβ\textsubscript{1-40} and Aβ\textsubscript{1-42}. These results show that the secretion of Aβ is influenced by neuronal activity. The treatment also increased and decreased β-CTF, respectively indicating that neuronal activity enhances β-secretase cleavage (Kamenetz et al 2003, Lesné et al 2005). Activating NMDA receptors sublethally (exposing cortical neurons of mice to concentrations of NMDA that did not lead to cell death) increased the production and secretion of Aβ. Furthermore a shift occurs altering the balance of generated transcripts from APP\textsubscript{695} towards KPI-APP (Kunitz-protease inhibitory domain). Application of AMPA, however, did not affect APP mRNA expression. Furthermore, the same shift in mRNA expression occurs in acute brain damage. Lesné and colleagues further showed that this shift in expression results from the Ca\textsuperscript{2+} influx after NMDA receptor activation (Lesné et al 2005).

The increased expression of KPI-APP was accompanied by an enhanced production of Aβ and sAPPβ, products of β- cleavage, Aβ\textsubscript{1-40} was elevated moderately whereas Aβ\textsubscript{1-42} was increased fivefold, and a reduction of p3 and sAPPα (products of α-cleavage) (Lesné et al 2005). Interestingly, Hoe et al report that Aβ\textsubscript{1-40} is greatly increased by 189% (Hoe et al 2009). Even though the sAPPβ increased the total amount of sAPP (sAPPα and sAPPβ) did not change. This indicates that NMDA receptor activation decreases the normally dominant α-secretase processing and increases β-cleavage (Lesné et al 2005).

Hoe et al (2009) indicate that this shift might be due to a change in APP trafficking. They state that levels of cell-surface expression of APP where significantly decreased by NMDA treatment compared to control treatment suggesting that NMDA activation results in endocytosis of cell-surface APP and thus to an increased possibility of β-cleavage (as β cleavage mostly takes place inside the cell whereas α-secretase cleaves predominantly at the cell-surface). Moreover they showed that APP increases NMDA receptor current density as opposed to a decreasing effect by Aβ (Hoe et al. 2009).

Earlier it was shown that an overproduction of APP led to a decrease in synaptic transmission (discussed by Kamenetz et al 2003). Kamenetz showed that the Aβ domain is sufficient to induce this depression by generating a chimeric β-CTF construct in which the Aβ-domain was replaced by the corresponding region in APLP2. While “normal” β-CTF is able to reduce synaptic transmission, the expression of the chimera did not result in a synaptic depression (Kamenetz et al 2003).

Summarizing, neural activity controls Aβ production by shifting the expression of mRNA which leads to a shift in α alternatively β-secretase cleavage (Hoe et al. 2009).

Furthermore, it was seen that, if levels are high enough, Aβ can also depress synaptic transmission onto nearby neurons indicating a paracrine function (Kamenetz et al 2003). From this Kamenetz proposed that Aβ may be a negative feedback regulator of synaptic activity as higher neuronal activity increases the production of Aβ which again leads to a
reduced synaptic function which decreases neuronal activity. Thus Aβ can prevent cells from excitotoxicity (Kamenetz et al 2003).

This is in agreement with the findings of Priller et al, where they investigated the effect of an APP knock-out on synaptic plasticity. They found that the excitatory synaptic transmission was strongly increased in neurons lacking APP mainly due to an increase in synapse numbers and to a lesser (or no) extent because of increased postsynaptic responsiveness or a change in presynaptic release parameters. The amplitude of AMPA- and NMDA-mediated ESPCs (excitatory postsynaptic currents) was heightened, indicating an increase in the strength of excitatory synaptic transmission, which can have multiple reasons. It could result from an increase in postsynaptic responsiveness, an alteration in presynaptic release parameters and a higher number of synapses. An indicator for postsynaptic responsiveness is the spontaneous mESPC. Priller et al found that mEPSC frequency was increased but not the amplitude, indicating that postsynaptic responsiveness and number of postsynaptic receptors are not altered in the absence of APP. Moreover, while the pool size (readily releasable pool, RRP) of synaptic vesicles per neuron was elevated, the synaptic and vesicular release probability which are both indicators for presynaptic release, remained unchanged. This points to a possible role of RRP in the increase in synaptic responses in APP knock-out autapses (Priller et al 2006).

However, quantitative immunohistochemical analysis shows that most probably an increase in the number of functional synapses per neuron is responsible for the increased synaptic transmission in APP ko slices. This is also in line with findings by Young and Selkoe in 2005 that neuritic outgrowth is enhanced in APP ko mice (discussed by Priller et al 2006) “This increase in synapses can explain the enhanced glutamate sensitivity and toxicity in mice lacking APP” (Priller et al 2006). Yet, these results were only found in young animals (3 weeks old), not adult animals. Furthermore, the observed effects could be due to one or more of the APP cleavage products. However, the involvement of AICD in this process can be excluded because neurons lacking APP still can produce AICD by γ-cleavage of APLP 1 and 2 thus hinting at a function of Aβ to reduce excitatory synaptic transmission by modulating the number of functional synapses. This mechanism would reduce glutamate excitotoxicity and cellular energy demands (Priller et al 2006). As APP is highly expressed in all kinds of neuronal injury (reviewed by Koistinaho 2007) eventually more Aβ is produced and can reduce excitotoxicity and prevent neuronal cell death.

The decrease of LTP can be explained by AMPA – and NMDA- receptor downscaling by an overload of Aβ resulting in decreased amount of AMPA- and NMDA receptors due to increased removal from the postsynaptic membrane and spine loss (reviewed by Parameshwaran 2008). Hsieh and colleagues also found that Aβ led to decreased surface
and synaptic AMPA receptors expression most likely because of endocytosis of the receptors and that endocytosis finally leads to spine loss (Hiesh et al. 2006). Moreover, Gu and co-workers show that Aβ reduces CaMKII in synapses which in turn prevents surface delivery of AMPA receptor and links Aβ causally to the loss of synaptic AMPA receptors (Gu et al. 2009). Furthermore, application of micromolar concentrations of Aβ₁₋₄₂ (a mixture of mature fibrils and protofibrils) attenuated AMPA-evoked neuronal firing whereas application of Aβ₁₋₄₀ had no effect (Parameshwaran 2007). As mentioned earlier, an attenuation of AMPA eventually leads to an impairment of memory formation.

The problem with most studies is that they work with Aβ overexpression as it is found in Alzheimer’s disease. Physiological amounts of Aβ might have a different effect than pathological amounts.

Studies using physiological amounts of Aβ₁₋₄₂ (in the picomolar range) show an enhancement in hippocampal LTP whereas nanomolar amounts attenuate LTP. Mice treated with picomolar concentrations of Aβ₁₋₄₂ showed better results in the Morris water maze, a spatial learning task that requires hippocampal function as compared to vehicle or scrambled Aβ₁₋₄₂ treated animals. They found the platform faster and after removal of the platform spent more time in the quadrant where the platform used to be. The mice also performed better in contextual fear memory (associative learning for which the hippocampal function is crucial) and showed more freezing behavior in response to the fear stimulus than vehicle or scrambled Aβ₁₋₄₂ treated animals. “This shows that the application of low doses of Aβ₁₋₄₂ cause a long-lasting increase in synaptic strength and enhance memory” (Puzzo 2008).

Moreover perfusion of brain slices from mice with picomolar amounts of Aβ₁₋₄₂ did not alter NMDA- or AMPA receptor mediated EPSCs amplitude nor did it change the current-voltage relationships of AMPA- and NMDA-receptor currents compared to controls. This indicated that the enhanced LTP was not achieved by postsynaptic changes of NMDA- and AMPA-receptor currents. However perfusion with Aβ₁₋₄₂ increased the posttetanic potentiation (PTP) via the α7-nACh receptors indicating that Aβ enhances neurotransmitter release during the tetanus. From these results Puzzo et al propose a model in which Aβ that is released due neuronal activity modifies the glutamate release with a mechanism dependent upon activation of α7-nACh receptors (Puzzo et al. 2008). This is further supported by observations that there is activation of α7-nACh receptors in different brain functions among others synaptic plasticity and memory and enhances transmitter release in several brain regions including the hippocampus. Additionally nicotinic activity at pyramidal neurons boosts LTP induction (described by Puzzo et al). Furthermore, Morley et al found increased ACh levels in mice treated with Aβ₁₋₄₂ as opposed to animals treated with saline. These findings strongly
indicate that Aβ1-42 itself might have an important modulatory function in synaptic plasticity and memory in the healthy brain (Morley et al. 2008).

The studies described above indicate that Aβ may have several distinct roles in the healthy individual. Low/physiological doses seem to enhance LTP and memory formation whereas higher doses lead to an attenuation of LTP and AMPA/NMDA receptor endocytosis. This could function as a protection mechanism against excitotoxicity. Moreover the roles of Aβ1-40 and Aβ1-42 may be different and also the aggregation state may play an important role.

3.2 Lipid metabolism

Another important physiological role of Aβ might be in the lipid metabolism and here mainly in the regulation of cholesterol and sphingolipids. The brain contains about 25% of total body cholesterol and is thus the most cholesterol-rich organ. As cholesterol is essential for development and maintenance of neural plasticity and function, cholesterol homeostasis, including synthesis, removal, storage and transport, must be strictly regulated (Hartmann 2006). HMGR (3-hydroxy-3-methylglutaryl-CoA reductase) is the rate-limiting enzyme in the biosynthesis of cholesterol and catalyzes the conversion of HMG-CoA to mevalonic acid. Drugs like statins completely block this enzyme resulting in lower cholesterol levels (reviewed by Buhaescu 2007).

After application of statins to primary hippocampal neurons and mixed cortical neurons, Aβ levels were reduced indicating that when cholesterol levels are lowered there is a reduced production of Aβ. Moreover, in guinea pigs treated with simvastatin it was shown that this reduction is reversible (Fassbender et al. 2001). Furthermore, a cholesterol-enriched diet increases cerebral Aβ levels in APP-transgenic mice and there is evidence that cholesterol enhances γ-secretase mediated Aβ production, thus shifting from the non-amyloidogenic to the amyloidogenic pathway (Grimm et al. 2007).

Moreover, in APP knock-out animals or in the absence of prenesilin, cholesterol levels increased. These increased levels could be reversed by administration of Aβ1-40 with HMGR-inhibition as the underlying mechanism. However, it is not known yet, whether Aβ1-40 is acting directly or indirectly on HMRG. These results led to the proposition of a negative feedback cycle: increased levels of cholesterol enhance APP processing and Aβ production. Aβ1-40 in turn inhibits HMGR activity, resulting in reduced cholesterol de novo synthesis and less APP processing. Furthermore the results indicate that Aβ has similar effects as statins (Hartmann 2006).

Another major group of lipids in cell membranes are sphingolipids, most of which are sphingomyelins (SMs). Together with cholesterol and glycosphingolipids, sphingolipids form the main component of lipid microdomains or rafts. It is assumed that SM levels are
controlled by Sphingomyelinase (SMase)-catalyzed degradation to ceramide and that Aβ\textsubscript{1-42} can interact directly with SMase and thus leading to a greater SM degradation (Hartmann 2006). However, it seems that SMs decrease Aβ production by inhibiting γ-secretase, leading to lower levels of Aβ production (Hartmann 2007). Moreover it was noticed that Aβ loses the capabilities mentioned above when it concentrations get too high, probably because toxic effects that work by other mechanisms increase. (Hartmann 2006).

It thus appears that physiological concentrations of Aβ are essential for lipid homeostasis and raft function by regulating cholesterol homeostasis (Hartmann 2006).

![Figure 3: A model of γ-secretase activity in lipid homeostasis. Cholesterol increases γ-secretase activity leading to an increase in APP processing and eventually to more Aβ\textsubscript{1-40} and Aβ\textsubscript{1-42} production. Aβ\textsubscript{1-40} inhibits HMGR, resulting in decreased cholesterol de novo synthesis. Aβ\textsubscript{1-42} downregulates Sphingomyelinin levels by stimulating nSMAse (neutral sphingomyelinase) and thus reducing the inhibiting effect that SM has on γ-secretase activity (from Grimm et al 2005).](image)

4. Physiological roles of AICD

4.1 Involvement in gene transcription

The APP-intracellular domain (AICD) is associated with gene transcription/nuclear signaling and may also be involved with regulation of apoptosis and calcium flux. (Giliberto 2008)

AICD is produced by cleavage by α- or β secretase, followed by γ secretase (described above) in membrane compartments upstream of the endoplasmatic reticulum.
(ER) and is dependent on presenilin. The AICD is a very labile fragment and can be degraded by the proteosome or the insuline degrading enzyme (Slomnicki 2008).

A putative role for AICD in transcription was suggested because of its similarities with the Notch intracellular domain (NICD) which is also produced from another class I transmembrane receptor (Notch) as a result of γ-secretase and is a component of a transcriptional complex (Slomnicki 2008).

AICD has at "least three functionally important motifs enabling interaction with several binding partners". These motifs are the YENPTY-motif which can be recognized by proteins from the FE65 family (promotes APP translocation to the plasma membrane and its processing), the Jip family and the X11 family (which prolongs the half-life of X11α and inhibits generation of Aβ probably by retarding endocytosis), the Shc family and the mDab1 proteins, Numb- and Numb-like proteins and clathrin (Slomnicki 2008).

The VTPEER motif can bind the 14-3-3γ protein which is highly expressed in brain, skeletal muscles and the heart. Moreover, the YTSI motif can be recognized by PAT1, a protein interacting with the APP tail 1 which may influence exocytosis and processing of APP. Thus interaction with PAT-1 seems to regulate basolateral sorting of APP.

<table>
<thead>
<tr>
<th>Motif</th>
<th>Binding partners</th>
<th>Function of Binding partners</th>
</tr>
</thead>
<tbody>
<tr>
<td>YENPTY</td>
<td>FE65 family</td>
<td>Promotes APP translocation to the plasma membrane (Sabo 1999)</td>
</tr>
<tr>
<td></td>
<td>Jip family</td>
<td>Role in axonal transport (Koushika 2008)</td>
</tr>
<tr>
<td></td>
<td>X11 family</td>
<td>Inhibits production of Aβ (Mueller 2000)</td>
</tr>
<tr>
<td></td>
<td>Shc family</td>
<td>Growth factor signaling (Ravichandra 2001)</td>
</tr>
<tr>
<td></td>
<td>mDab1 proteins</td>
<td>Regulates axonal extension, targeting and branching in the hippocampus (Borrell 2007)</td>
</tr>
<tr>
<td></td>
<td>Numb- and Numb-like proteins</td>
<td>Increase cell proliferation (Johnson 2003)</td>
</tr>
<tr>
<td></td>
<td>Clathrin</td>
<td>Mediates endocytosis of cell surface components (reviewed by Cavalli 2001)</td>
</tr>
<tr>
<td>VTPEER</td>
<td>14-3-3γ protein</td>
<td>Adapter protein, promote cell proliferation (Hong 2008)</td>
</tr>
<tr>
<td>YTSI</td>
<td>PAT1</td>
<td>Regulates basolateral sorting of APP (Slomnicki 2008)</td>
</tr>
</tbody>
</table>

Table 1: AICD motifs and binding partners.

The most attention has been given to AICD-FE65 interaction. FE65 possesses 3 protein-protein interaction domains, one WW domain and two PID domains (Slomnicki 2008).

Four different mechanisms of AICD signal transduction have been suggested. The first mechanism states that AICD recruits FE65 and changes its conformation. After that FE65 translocates into the nucleus and binds to TIP60 (Cao et al 2004). The second
mechanism suggests that AICD and FE65 translocate into the nucleus independently and form a ternary complex with TIP60 in the nucleus (Reviewed by Müller 2008). In the third mechanism APP recruits TIP60 through FE65 which results in a phosphorylation, stabilization and activation of TIP60 by CDK. The TIP60-FE65 complex moves into the nucleus. This is a γ-secretase independent mechanism (Hass et al 2005).

However, the most approval has been given to the fourth mechanism. In this model the AICD-FE65 complex forms at the membrane and translocates into the nucleus. Once inside the nucleus TIP60 enters the complex forming the transcription factor (Reviewed by Müller 2008).

Some AICD target genes found are GSK3β (glycogen synthase kinase) which is involved in tau phosphorylation, Neprilysin which is responsible for Aβ degradation and p53, a tumor suppressor gene. P53 is directly involved in the transcriptional activation of pro-apoptotic effectors such as caspase 1 and the oncogene bax. p53 thus plays a potential role in cell death signaling/apoptosis. Interestingly GSK3β interacts with p53 and promotes the apoptotic response. (Müller 2008)

Furthermore, AICD upregulates APP gene expression in a positive feedback manner. It also upregulates the gene encoding for BACE, however no differences were found in genes for the γ-secretase complex PSEN1 or ADAM10 which is thought to be one of the α-secretases. This led von Rotz to the conclusion that full-length APP not only regulates its own expression via AICD, but also influences the expression of several genes that are involved in its processing and cellular function (von Rotz 2004).

However, Giliberto showed that AICD probably does not regulate basal gene expression because no obvious correlation between AICD mRNA and gene levels was observed between AICD transgenic mice and control littermates. However, this does not mean that AICD is not involved in gene expression (APP, p53, KA11, NEP), it might regulate transcription of yet unidentified genes. They conclude that “AICD might have a transcriptional function in either a small subset of forebrain neuronal cells or under specific signaling or stressful conditions”. They assume that AICD may lower the resistance to toxic and apoptotic stimuli and could thus work as a possible cell-death mediator and because of this could have a detrimental effect (Giliberto et al 2008). However, as mentioned earlier AICD is a very labile and huge amounts would be necessary to reach a detrimental effect. Additionally, these conflicting findings could be due to differences in cell cultures or mouse models.
<table>
<thead>
<tr>
<th>Target gene</th>
<th>Up-/downregulation</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>APP</td>
<td>↑</td>
<td>Precursor for Aβ, function see part 2</td>
</tr>
<tr>
<td>BACE</td>
<td>↑</td>
<td>Cleaves APP at the β-cleavage site</td>
</tr>
<tr>
<td>Neprilysin</td>
<td>↑</td>
<td>Aβ degradation</td>
</tr>
<tr>
<td>p53</td>
<td>↑</td>
<td>Tumor supressor gene, mediates apoptosis</td>
</tr>
<tr>
<td>GSK3β</td>
<td>↑</td>
<td>Tau phosphorylation, can interact with p53</td>
</tr>
</tbody>
</table>

Table 2: AICD target genes and function, ↑: upregulation. The function represents the most important function in the context of this paper, there might be other functions for each gene.

Summary and Conclusions
The studies discussed above give a lot of evidence for a putative physiological roles of APP, Aβ and AICD. APP is amongst other factors important for cell-migration and is hereby involved in wound healing and the migration of neuronal precursor cells into the cortical plate in the developing brain. Furthermore, it increases the NMDA receptor activity by decreasing the NR2B internalization. Aβ on the other hand decreases neural activity and it was suggested by Kamenetz et al (Kamenetz et al 2003) that it might play a role in a negative feedback mechanism to prevent cells from excitotoxicity. As APP is increased by traumatic brain injury it leads to more excitation of cells but also to more Aβ production which again lowers the excitation. It could thus work as a “brake-mechanism”. However, it was also shown that this inhibition of neuronal activity is only achieved by large amounts of Aβ, as it is found in the AD brain where it reaches pathological quantities. In physiological amounts, Aβ leads to an enhancement of hippocampal LTP and consequently improves learning and memory. Moreover, Aβ works in a negative feedback manner in lowering elevated cholesterol levels. However, more research is necessary to clarify conflicting findings. It is difficult to compare the results of many studies because different amounts of Aβ, different forms (Aβ1-40 or Aβ1-42) and different aggregation states are used. Future studies on the physiological role of Aβ should not use overexpression of APP or Aβ. From these results it should be clear that drugs designed to inhibit Aβ formation such as γ-secretase or β-secretase inhibitors should be handled with care as they might have detrimental effects for patients. It could lead to more excitotoxicity and even result in impairment of memory which is the opposite of the desired effect. Moreover it could negatively affect the cholesterol levels. An inhibition of γ- or β secretase would also lead to a decreased production of AICD. The AICD seems to play an important role in gene transcription, it does for example increase the transcription of the APP gene and the gene
encoding for BACE, thus regulating its own expression. It was also suggested that BACE lowers the resistance of cells to apoptotic stimuli thus making it more prone to cell death.

Concluding, the findings described above strongly indicate physiological functions for APP, Aβ and AICD, which should also be kept in mind for the development of new medication against Alzheimer’s Disease. Future research should focus more on physiological amounts of Aβ. More needs to be known about the underlying function in which Aβ impairs or rather enhances memory formation and which roles Aβ_{1-40} and Aβ_{1-42} play exactly. On a highly speculative basis Aβ_{1-40} could even be utilized as a cognitive enhancer. Moreover it might be used as medication in a statin-like manner for patients suffering from hypercholesterolemia. However, a lot more research is necessary before these options become feasible.

References:


Hong SW, Qi W, Brabant M, Bosco G, Martinez JD (2008) Human 14-3-3 gamma protein results in abnormal cell proliferation in the developing eye of Drosophila melanogaster. Cell Division 3:2


Young-Pearse TL, Chen AC, Chang R, Marquez, Selkoe D (2008) *Secreted APP regulates the function of full-length APP in neurite outgrowth through interaction with integrin beta I.* Neuronal Development 3:15


- 21 -