

Strategies to Diminish the A Load in Alzheimer's Disease

A.C. Cuello^{1,2,3,*} and K.F.S. Bell¹

¹Departments of Pharmacology & Therapeutics, ²Neurology & Neurosurgery and ³Anatomy & Cell Biology, McGill University, Montreal, Quebec, Canada, H3G 1Y6

Abstract: Striking advances have been made in recent years toward potential therapies for Alzheimer's disease. Alzheimer's disease, which is the leading cause of dementia in the elderly, is pathologically defined by the presence of amyloid plaques, composed of the amyloid-beta protein, and neurofibrillary tangles. The amyloid pathology has been associated with decreased synaptic plasticity and neurodegeneration, thereby explaining the visibly decreased cognitive function and evident dementia. Subsequently, a large number of studies have been launched, which attempt to disrupt the progression from A aggregation to plaque formation. These studies have involved the use of beta-sheet breakers, secretase inhibition, immunotherapy and anti-inflammatories, the most notable findings of which are discussed in this review.

Keywords: Alzheimer's disease, amyloid-beta protein, amyloid precursor protein, amyloid plaques, secretase, inflammation, immunotherapy, beta-sheet breakers, transgenic

HALLMARKS OF THE ALZHEIMER'S DISEASE MOLECULAR PATHOLOGY

The amyloid beta (A β) and tau proteins comprise the two main molecular components of the Alzheimer's disease (AD) pathology. A β peptides are produced as a result of the abnormal cleavage of the Amyloid Precursor Protein (APP) (for review see [1,2]) and as yet, have not been linked to any definitive biological role. The tau protein however, is a cytoskeletal protein which is involved in the stabilization of microtubules, *via* its microtubule binding domain [3-6]. Two forms of the A β peptide (A β ₁₋₄₀ and A β ₁₋₄₂) have been shown to be involved in the Alzheimer's molecular pathology. The latter form, A β ₁₋₄₂, tends to polymerize and subsequently, aggregate more rapidly, leading to an accelerated formation of amyloid plaques typically located in the cerebral cortex and hippocampus [7,8]. All 6 of the known tau isoforms possess either a 3 or 4 repeat motif within their microtubulin binding domain and are all capable of generating paired helical filaments (PHFs), following abnormal phosphorylation [9-11]. These tau-generated PHFs constitute the core protein of the so called neurofibrillary tangles (NFTs), which along with amyloid plaques comprise the two main hallmark lesions of the AD pathology. NFTs have also been observed in a large number of other diseases, which are referred to as "tauopathies" (for review see [12-14]).

Despite continuing debates regarding the involvement and association of these two lesions in the AD pathology, most would agree that APP dysmetabolism and the resultant generation of A β peptides are central components of the disease's pathogenesis. This is supported by the increased A β peptide production seen in patients with early onset or familial AD (FAD), which generally result from mutations in

either the APP or presenilin 1 & 2 genes [15,16]. Unlike the early onset or familial forms of AD, sporadic AD, which accounts for the majority of all AD cases, has a large number of both genetic and non-genetic risk factors. However, despite the age of onset or genetic traceability, all three disease forms exhibit identical neuropathological lesions and cognitive deficits, a commonality, which led to the "Amyloid cascade" hypothesis.

This hypothesis suggests that a direct correlation exists between the impaired cognitive function seen in AD patients and the presence of amyloid plaques in the brain, as illustrated in (Fig. 1). Subsequently, a great deal of effort has been directed toward gaining a better understanding of the processes involved in both the generation and extracellular aggregation of A β peptides in the CNS. These investigations have identified three membrane spanning APP isoforms, namely APP_{695,751} and 770, all of which contain the A β domain at a location which is partially exposed to the extracellular space [17]. A number of secretases exist, which act on specific cleavage sites along the APP. TACE₁ or α -secretase, for example, cleaves the APP at a site within the A β domain. By doing so, it effectively precludes the formation of the A β peptide and instead gives rise to the "non-amyloidogenic" protein, soluble APP, see (Fig. 2). A β formation requires the activity of two distinct convertases, which cleave at two separate sites along the APP. Cleavage at the amino terminal domain of the A β peptide is dependent on the α -secretases, which have been identified as BACE₁ and BACE₂ (α -APP converting enzymes), the former of which is considered to be more relevant to A β peptide formation [18]. Cleavage at the carboxy terminal site is made by the γ -secretase(s). The identity of the γ -secretase has, as yet, not been indisputably defined, however at present, the presenilin molecules appear to be the best candidates [19]. The presenilins possess aspartyl protease enzyme properties and appear to acquire their γ -secretase activity by forming functional complexes with other molecules (for reviews see [20,21] and see Fig. 4). This would make sense given that FAD patients, with muta-

*Address correspondence to this author at the Department of Pharmacology and Therapeutics, McGill University, 3655 Promenade Sir-William-Osler, Room 1210, Montreal, QC, H3G 1Y6 Canada; Tel: (514)398-3618; Fax: (514)398-8317; E-mail: claudio.cuello@McGill.ca

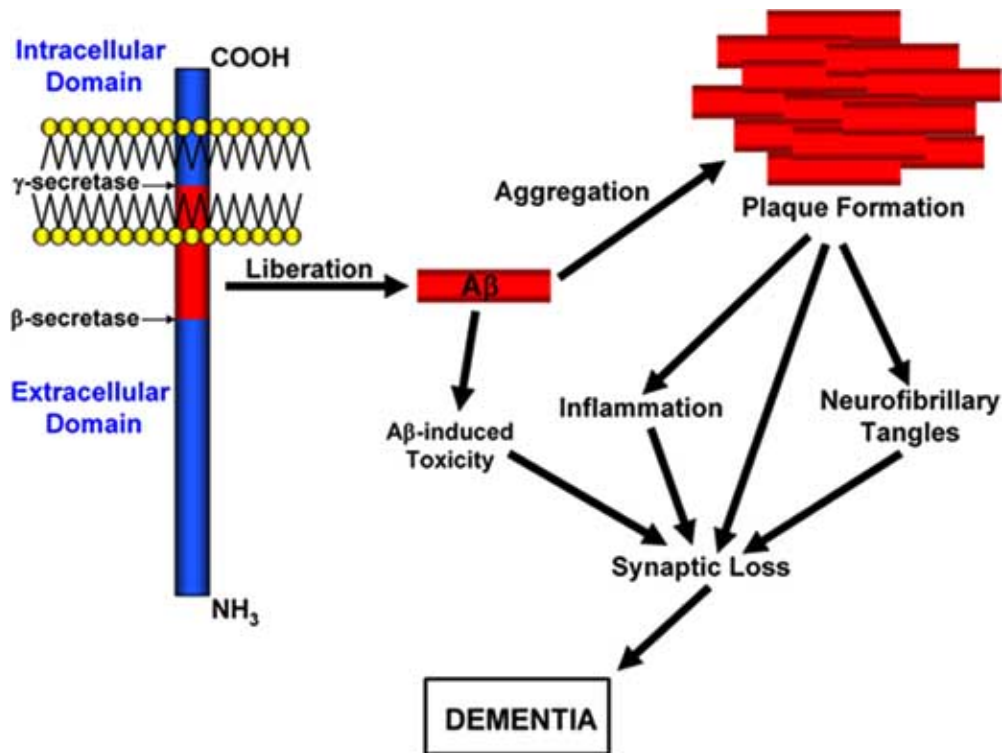


Fig. (1). Schematic representation of the main features of the Amyloid Cascade hypothesis.

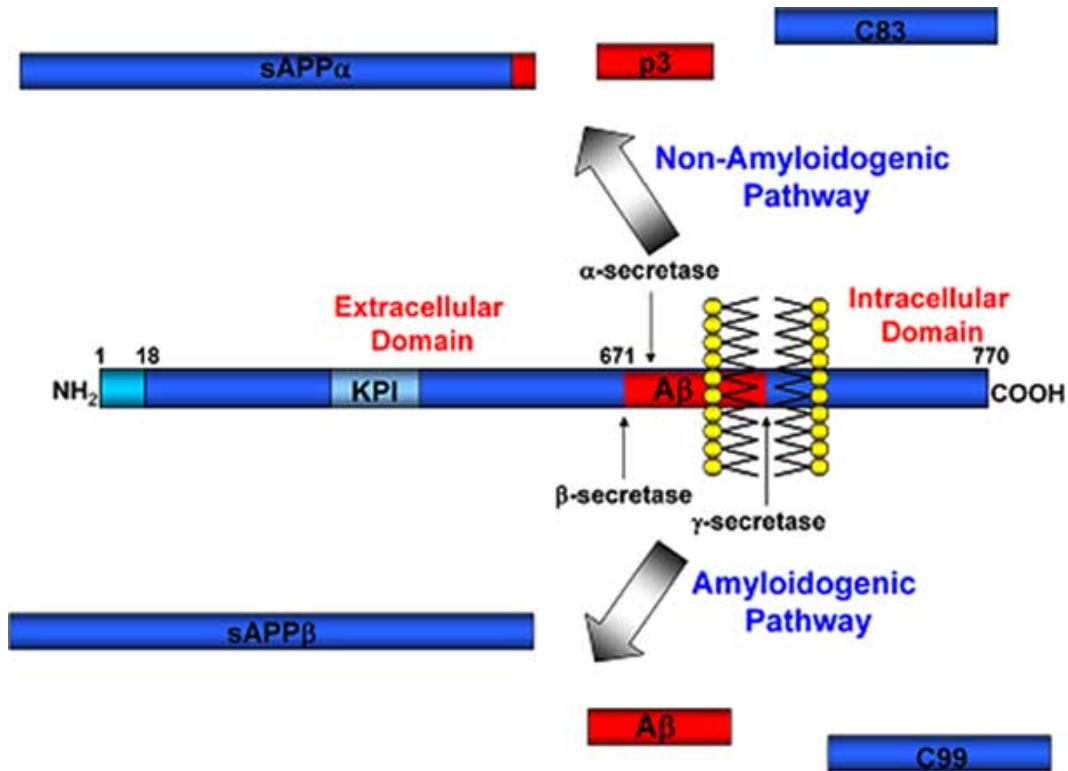


Fig. (2). Pathways involved in the metabolism of APP and resultant cleavage products.

tions in either the PS1 or PS2 genes, typically display a “gain of function” in their γ -secretase activity. In contrast, APP mutations lead to an increased production of A₁₋₄₂ fragments or A₁₋₄₂ specifically. Mutations in these three genes are similar, however, in that they all accelerate A₁₋₄₂ aggregation

and subsequent plaque formation (for review see [15,22]). Currently, the debate centers around which form of A₁₋₄₂ is responsible for eliciting a neurotoxic effect: aggregated A₁₋₄₂ found in true amyloid plaques, or the earlier monomeric, polymeric and fibrillar forms (for reviews see [23]).

These discussions are of particular relevance to synaptic attrition, which has been clearly linked to the visible cognitive deficits seen in AD patients, as was largely shown by Terry and collaborators [24] (for review see [25,26]). What is unclear however, is whether this synaptic attrition results from an A β -induced toxicity or alternatively as a “synaptosis” due to a PHF and NFT-induced cytoskeletal dysfunction. The use of transgenic animal models displaying increased amyloid deposition has shown that an increased A β burden causes synaptic loss and dystrophic neurite formation [27-30], evidence which supports the “amyloid cascade hypothesis”. Another hallmark of the AD neuropathology, which has received considerably less attention than other markers (and perhaps not rightfully so), is the visible inflammatory component (for reviews see [31-33]). Many authors tend to regard inflammation and oxidative stress as a secondary component of the pathological evolution, however Perry, Smith and collaborators [34], amongst others [35,36], propose that oxidative stress may in fact be an initiating disease factor.

Despite this inflammatory involvement, the majority of research continues to focus on the A β peptide, since APP dysmetabolism and A β aggregation are consistently regarded as the crucial, if not causative, components of the AD pathology. Much of the research investigating the inhibition of A β production and aggregation, as well as plaque breakdown, has been greatly facilitated by the development of transgenic animal models expressing AD-like pathology.

In this review, we summarize some of the more notable approaches towards understanding the neuropathology of Alzheimer's disease, and indicate some of the possible therapies that may arise as a result of this new knowledge.

REVERSING THE A β FIBRILLIZATION AND AGGREGATION

A plausible evolution of the extracellular A β pathology would be that neurons within the cerebral cortex and hippocampus release soluble monomeric forms of the A β peptide. These monomeric peptides would then form successive dimeric and oligomeric fibrils, which would aggregate together into diffuse plaques prior to forming true amyloid plaques, as is shown in Fig. 3. This process of nucleation appears to be accelerated by both pre-existing fibrils and the presence of seeding amyloid material [37]. True amyloid plaques contain a β -pleated sheet core, which is both thioflavin-S and Congo red positive, and is typically surrounded by a corolla of less compact, diffuse A β material. Proteins, reactive astroglia, microglia and even neuronal processes (dystrophic neurites) can also be seen in the periphery of these “true” or mature “neuritic plaques”.

Several interpretations of the importance of various components involved in A β dimerization and subsequent aggregation into the β -pleated sheet conformation currently exist. While A β polymerization and fibrillization are known to be dependent on pH, temperature and time [38], additional studies have shown that other agents such as heparan sulphates, glycosaminoglycans, short length peptides, Congo Red analogues and even certain metals, can also have an effect on A β fibrillization (for review see [39]).

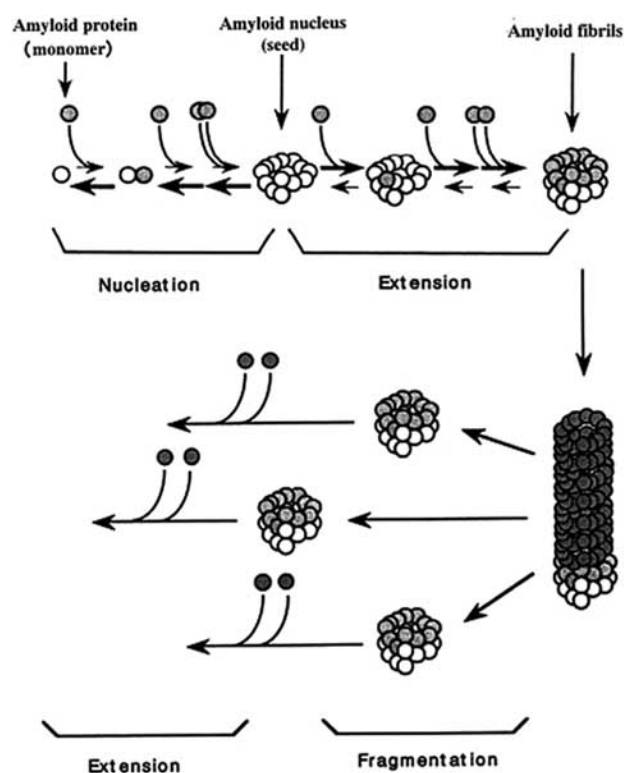


Fig. (3). Nucleation-dependent polymerization model of amyloid fibril formation (reproduced with permission, from Xing and Higuchi, 2002).

Heparan Sulphates & Glycosaminoglycans

An early study by Kisilevsky *et al.*, demonstrated that the parenteral application of small molecular weight, anionic sulphonates and sulphates was capable of inhibiting experimental amyloidosis in the spleen [40], a finding which prompted the authors to suggest the approach as a potential treatment for AD. Similarly, injections of low molecular weight heparins (such as enoxaparin and dalteparin) were also shown to arrest the progression of amyloidosis in a comparable peripheral model [41]. Interestingly, Hanin, Cornelli and collaborators [42,43], found that low molecular weight glycosaminoglycan administration can effectively reduce the impact of intracerebral A β application in the CNS. Furthermore, these agents were also found to lessen the cholinergic lesions resulting from the application of the AF64A neurotoxin [44,45]. An additional noteworthy approach has been the development of a series of low molecular weight anionic compounds which mimic the glycosaminoglycan moieties of proteoglycans. These compounds elicited both a protective and anti-fibrillogenic effect against A β -induced cytotoxicity *in vitro* [46].

Short Peptides

An *in vitro* study by Soto and collaborators found that short linear peptides of the 17-21 amino acid range (LVFFA) effectively inhibited A β amyloid formation and, furthermore, induced a disaggregation of pre-formed fibrils upon incorporation of proline residues [47]. The investigation was based

on the assumption that the central hydrophobic region of A is responsible for the conformational changes which lead to A aggregation. Proline residues were incorporated into the peptides as proline has a low propensity to form β -pleated sheets [48] and A homologous peptides containing proline residues are non-amyloidogenic [49]. More recent *in vitro* work by the same group demonstrated that the covalent modification of certain "beta-sheet breaker" peptides, with naturally occurring polyamines, is able to increase the peptide's permeability through the blood brain barrier, while preserving its ability to inhibit A fibrillogenesis [50]. Simultaneously with Soto's initial communication, Tjernberg and collaborators proposed that peptides containing an A₁₆₋₂₀ (KL₁₆VFF) sequence can bind full-length A and subsequently prevent its assembly into amyloid fibrils. This is logical since the A₁₆₋₂₀ (KL₁₆VFF) region represents the required binding sequence for A polymerization and fibril formation [51]. The authors have shown that the amino acids Lys₁₆, Leu₁₇, and Phe₂₀, appear to be essential to A binding, as the inhibition of these residues effectively reduces A fibril formation [51]. Thus, by screening combinatorial pentapeptide libraries composed of D-amino acids, the study was able to identify ligands capable of preventing A fibrillization, as well as several protease-resistant ligands [52]. These protease resistant ligands contained a general motif of phenylalanine in the second position and leucine in the third position, and were able to prevent the formation of amyloid-like fibrils [52]. Taking advantage of the model proposed by Tjernberg *et al.*, Akikusa and collaborators recently developed an *in vitro* rapid assay system containing the immobilized KL₁₆VFF minimal aggregation motif, in an effort to identify putative A "breakers" [53].

The degree to which these synthetic peptides are methylated and amidated appears to have a bearing on their ability to inhibit A fibrillization *in vitro* [54,55]. The enantiomeric state of amino acids, specifically replacing L-enantiomers with D-enantiomers, has also been investigated with respect to the efficiency of A fibrillization inhibition. Chalifour *et al.* found the D-enantiomers of five specific peptides, KL₁₆VFFA, KK₁₆L₁₆VFFA, KF₁₆VFFA, KIV₁₆VFFA, KVV₁₆VFFA, to be more active as inhibitors in an *in vitro* fibrillogenesis assay than their L-enantiomer counterparts [56].

Congo Red

Congo Red (CR) is a classic histological marker used to identify fibrillar amyloid material. Because of this characteristic, CR and CR analogs have been proposed as potential inhibitory agents of both A fibrillization and A-induced toxicity in certain *in vitro* models [57]. These effects were attributed to the stabilization of monomeric A by CR [58]. Bi-pyridinyl and other CR analogues, in which sulphate groups are replaced by carboxylates, have shown a similar ability to inhibit A fibrillization with dissociation constants below 1 μ M [59,60]. Unfortunately, no animal data currently exists to support a therapeutic role for CR or CR derivatives in AD-like pathology.

Metals

Several metals, most notably Cu and Zn, have been shown to rapidly induce the fibrillar organization of A

[61,62] by forming intermolecular bridges with the histidine residues of neighboring A peptides [63], thus, prompting the thought that Zn chelating agents might be significant in preventing A aggregation. Clioquinol is a chelating antibiotic, which effectively sequesters both Cu and Zn. The drug, however, has been retired from most national pharmacopeias due to its tendency to provoke subacute forms of blindness, muscle weakness and numbness and tingling sensations in the lower extremities [64]. Despite these deleterious effects, a number of investigators continue to advocate the use of clioquinol at doses below toxic levels. One study, which investigated the effect of orally administering clioquinol to transgenic mice (APP₂₅₇₆) for 9 weeks, found a marked reduction in A levels within the brains of treated mice [65]. In fact, a small, open clinical trial, involving the administration of clioquinol to AD patients, found slight improvements as early as 3-weeks post administration [66]. A larger clinical trial is currently underway, from which results are soon expected [67]. Other antibiotics, such as Rifampicin, have also shown A disaggregation properties [68,69], however, no route to experimental therapeutics has as yet been found.

INHIBITING THE PRODUCTION OF A

Stimulation of β -Secretases

With this improved understanding of the APP processing pathway and of the cleavage enzymes associated with the production of A, a number of novel and potentially therapeutic targets have been proposed, which would alter the molecular processing of APP in order to favour the production of non-amyloidogenic fragments. Alpha-secretase has been reported to be regulated by protein kinase C (PKC) [70,71] and is assumed to be a member of the disintegrin and metalloprotease family of enzymes, including TACE (tumor necrosis alpha converting enzyme) and ADAM-10 [72]. Increased activation of the β -secretase presents an obvious therapeutic target, as its activity precludes the production of A (see Fig. 2). Despite this, there are currently no widely accepted β -secretase activators. Kozikowski *et al.* [73] have described a series of benzolactams which are capable of stimulating β -secretase activity (in varying degrees) *in vitro* in cell lines obtained from AD patients. The side chain identities within these series of compounds were found to have an important impact on both membrane translocation and PKC activation. However, their potential utility remains limited since the compounds are only effective at very high concentrations, at which they could potentially elicit tumor-promoting activity.

Certain compounds, including acetylcholinesterase (AChE) inhibitors, are capable of facilitating β -secretase activity in both an indirect and non-specific manner. AChE inhibitors, which prevent the breakdown of the neurotransmitter acetylcholine, are commonly administered to symptomatically treat AD. The most widely prescribed AChE inhibitors are Tacrine (Cognex), Donepezyl (Aricept), Rivastigmine (Exelon) and Galantamine (Riminyl), all of which vary in their pharmacodynamics, propensity to provoke the "cholinergic syndrome" and relative toxicity. The rationale for their application in AD lies in their ability to boost residual ACh levels at the synapse, which become heavily compromised in the basal forebrain [74-76], likely as

with aspartyl proteases, should therefore inhibit γ -secretase function [91]. Interestingly, the photoactivation of these compounds results in their covalent binding with PS, which further enforces the idea that the PS do indeed contain the catalytic function of the γ -secretase [92]. A number of compounds, some of which mimic the transition state and some of which do not, have elicited an inhibitory action on the γ -secretase site *in vitro* [93]. One of the earliest compounds which reportedly mimicked the aspartyl protease transition state was N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenyl glycine t-butyl ester. This compound, referred to as DAPT [94], was used in parenterally administered transgenic mice bearing the human APP_{V717F} transgene [95], and displayed a significant, dose-dependent reduction of A β levels in the brain, with a peak efficacy attained 3 hours post-treatment and a consistent, reduced A β burden for up to 18 hours. These investigations demonstrate an acute effect of the putative γ -secretase inhibitor in young (3-4 months) mice at a stage in which no amyloid plaques are present. A more recent study [96] replicated and expanded the study in Tg2576 mice [97] and found that DAPT administration induced a dose-dependent decrease in both plasma and CSF A β levels in young plaque-free mice as well as older plaque-bearing mice. While no consistent diminution in measurable A β levels was noticed in the brains of the aged (plaque-bearing) mice [96], the authors suggest that the decreased plasma and CSF A β levels likely indicate an inhibition of CNS A β production.

The multifaceted role of γ -secretase poses an overwhelming obstacle for the development of a clinically effective inhibitor. The PS1/ γ -secretase complex is known to participate in a number of important regulatory mechanisms, as is supported by studies involving PS1 $-/-$ KO mice, which display embryonic lethality, marked skeletal malformations and CNS abnormalities similar to those observed in Notch $-/-$ mice [98]. Furthermore, γ -secretase is responsible for the functional cleavage of an intracellular domain of the membrane bound tyrosine kinase, ErbB-4 [99], a process which has been shown to be blocked by γ -secretase inhibitors. Finally, certain products of γ -secretase cleavage may have a required or beneficial physiological role, for example the APP intracellular domain (AICD) has been reported to aid in intracellular Ca $_2^{++}$ homeostasis, a function which would be compromised by γ -secretase inhibition [100].

Lewis and collaborators analyzed a number of potential γ -secretase inhibitors by assessing the cleavage products in a human cell line stably expressing both APP and the truncated Notch $_1$ receptor fragment, Notch-Delta-E, via Western Blot analysis [101]. The study found a close correlation between the degree of inhibition for both substrates, suggesting that the Notch and APP cleavage sites cannot be easily dissected apart [101]. Kornilova, Das and Wolfe [102] investigated the displacement of a photo-affinity bound probe from the active site of the PS heterodimer, and found that they could then define the differential modalities of a variety of reported γ -secretase inhibitors. Thus, as summarized in Table 1 (as adapted from Kornilova *et al.*), it appears possible that γ -secretase inhibitors act by containing the same transition state type, affecting the allosteric conformation, mimicking the substrate or alternatively, and as in the case of coumarin derivatives, may not physically act as a γ -secretase inhibitor

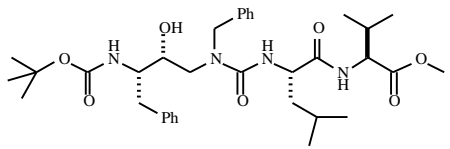
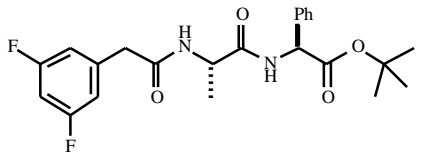
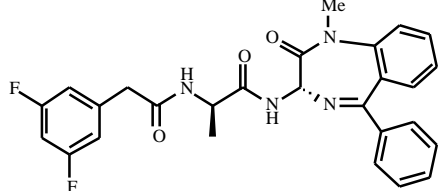
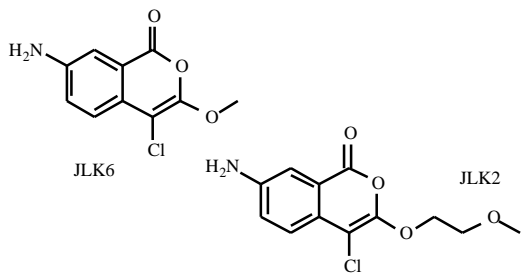
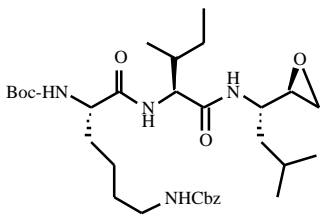
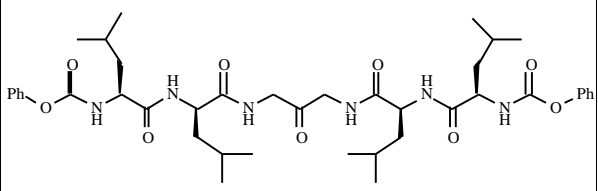
in cell free-systems. The latter possibility, is particularly interesting as coumarins have been reported to inhibit the γ -secretase site *in vitro* without affecting Notch cleavage [103], an aspect which has been confirmed by others [104]. A number of non-competitive γ -secretase inhibitory compounds have also been reported by Tian and collaborators [105], which suggests that unidentified molecular mechanisms resulting in the prevention of A β synthesis may exist. More recently these authors elegantly suggested a physical separation of the sites for substrate binding and catalysis, with binding of the transition state analogs to the catalytic site and not to the substrate site [106]. The search for γ -secretase inhibitors with differential mechanisms of action (i.e. transition state, allosteric conformation, mobilization of substrate, intervention of γ -secretase-associated protein) might render compounds which would eventually strike an appropriate balance between inhibition of A β production and maintenance of other physiologically relevant γ -secretase products. In this regard, a recent publication by Phiel *et al.* [107] has brought rather exciting and thought provoking *in vitro* evidence that the lithium-induced inhibition of the kinase GSK $_3$ (and not GSK $_3$) renders a pronounced diminution in the generation of A β fragments in APP₆₉₅ stably transfected cell lines. A similar effect was achieved with the GSK $_3$ inhibitor kenpaullone but not with a CDK $_5$ inhibitor. RNA interference assays, which blocked synthesis as well as over-expression of both GSK $_3$ and GSK $_3$, suggested that GSK $_3$ may have a stimulatory role in the generation of A β , while GSK $_3$ may have an inhibitory role. These manipulations had little impact on the generation of NICD fragments, thus suggesting a mechanism of action interfering with presenilin associated proteins. Interestingly, the application of lithium was found to provoke similar effects in primary cortical cells of Tg2576 mice as well as a decreased A β brain content when applied *in vivo* at early, plaque-free stages. These findings are additionally relevant as lithium has been reported to block tau aggregation via GSK $_3$ inhibition [39].

Inhibition of γ -Secretase

The γ -secretase site is most relevant for the AD pathology since APP mutations around this cleavage site, such as the Swedish double mutation, result in a gain of function, causing increased generation of A β [108]. Two putative γ -secretases have been identified and cloned and are referred to as BACE $_1$ [109] and BACE $_2$ [110], the former of which appears to be most significant for A β generation (reviewed in [111]). In recent years, the design of potential γ -secretase inhibitors has received a great deal of attention. One of the main attractions of this target was found when BACE $_1$ $-/-$ KO mice had no significant CNS pathology [112,113], while A β formation was prevented in the absence of a compensatory action of BACE $_2$ [112,113]. On the other hand transgenic overexpression of BACE $_1$ significantly increases steady-state levels of A β in the CNS [114] while overexpression of BACE $_2$ does not augment A β formation [115-117].

APP cleavage at the γ -secretase site is an upstream and presumably intracellular event within the Golgi and endoplasmic reticulum compartments [109]. Therefore, in contrast to membrane-acting γ -secretase inhibitors, drugs targeting γ -secretases should act intracellularly [109]. The

Table 1. Chemical Structures, Inhibition Properties, and Displacement Abilities of Various γ -Secretase Inhibitors (Reproduced with Permission from Kornilova *et al.*, 2003)

Compound	Structure	In cells	Cell-free	Displacement	Type
III-31-C		0.2 μ M	10 μ M	YES	Transition state
DAPT		20 nM	10 nM	YES	Non-transition state
Compound E		0.3 nM	3 nM	YES	Non-transition state
Isocoumarins		80 μ M	>200 μ M	NO	Non- γ -secretase
D-Helical peptide 294	Boc-D-Val-Gly-Aib-D-Val-D-Val-D-He-Aib-D-Thr(OBn)-D-Val-Aib-OMe	3 μ M	0.1 μ M	NO	Substrate mimic
Epoxide		20 μ M	20 μ M	YES time-dependent	Irreversible
(Z-LL) ₂ -ketone, a SPP inhibitor		>100 μ M	30 μ M	YES	Aspartyl protease inhibitor

difficulties in obtaining therapeutically effective γ -secretase inhibitors are common to all similar aspartic proteases. Hong *et al.*'s recent identification of the x-ray crystallographic structure of BACE₁ complexed with the peptide inhibitor

OM99-2 has greatly facilitated new approaches for rational γ -secretase inhibitor drug design [118]. From this identification, the group was then able to identify a number of structure-based designed compounds with potential inhibitory

effects of β -secretase activity [119]. Furthermore, utilizing a peptide combinatorial library, the subsite specificity within BACE has also now been mapped by Turner and collaborators [120]. Tung *et al.* [121] have described a number of substrate-based peptide inhibitors of β -secretase and recently Elan Pharmaceuticals [122] have utilized a hepta-peptide as a base to generate smaller peptide-mimetics. The C- and N-terminal portions of the framework molecule were then substituted in a reiterative manner in order to test their ability to block A β production at the β -secretase site *in vitro* [122]. A compound coded 38 displayed the highest activity with an IC₅₀ of 4 μ M on APP_{HEK-293} cells (see Fig. 5) and [122]).

STRATEGIES FOR A β -IMMUNOTHERAPY

In recent years the possibility of diminishing the A β burden by applying immunological approaches has received much attention. Three different immunotherapeutic strategies are currently under investigation; these are 1) active immunization with full length synthetic A β , 2) active immunization with a specific A β epitope, conjugated to a hapten protein carrier and 3) passive immunization with exogenously produced monoclonal antibodies (Mabs) directed against A β .

Preclinical Findings

Solomon *et al.* were the first group to demonstrate that the application of A β -specific Mabs against residues 1-28 can both prevent A β self-aggregation and preserve A β solubility [123]. Furthermore, a monoclonal anti-A β ₁₋₁₆ antibody was able to restore the solubility of preformed A β aggregates as well as elicit a protective effect against A β -induced neu-

rotoxicity [124]. Following these initial *in vitro* experiments, Elan Pharmaceuticals actively immunized PDAPP mice with AN-1792 (full length synthetic A β ₁₋₄₂ plus QS-21), and observed a subsequently increased level of anti-A β antibodies within the murine serum and significantly decreased levels of amyloid plaque burden, neuritic dystrophy and astrogliosis [125]. Elan went on to passively immunize PDAPP mice with the A β -specific Mabs, 10D5 and 3D6, which effectively reduced plaque burden by over 81% and 86% respectively. *Ex vivo* assays of serial brain sections from the same animals, performed in the presence of 10D5, displayed drastically reduced plaque levels as well as the presence of internalized A β within phagocytic vesicles of surrounding microglia [126], suggesting that F_c receptor-mediated phagocytosis may in part be responsible for peptide degradation. The capability of exogenously applied anti-A β Mabs to remove cortical amyloid aggregates has been dramatically illustrated by direct microscopic imaging of *in vivo* antibody-induced plaque clearance [127]. Important evidence for the “proof of principle” of immunization in experimental therapy was demonstrated by two different studies investigating distinct transgenic mouse models, both of which not only demonstrated a true amyloid lowering effect, but also an accompanied improvement in behavioural performance [128,129]. An effective A β immunization can be achieved by applying a variety of routes for the administration of the immunogen, including nasal application of the vaccine [130]. Frangione and collaborators raised the possibility that the use of A β ₁₋₄₂ as an immunogen may facilitate seeding and fibrillization (see above and Fig. 3) thus, potentially worsening the AD pathology [131]. These authors therefore developed a non-amyloidogenic non-toxic shorter A β homologue, K₆A β ₁₋₃₀.

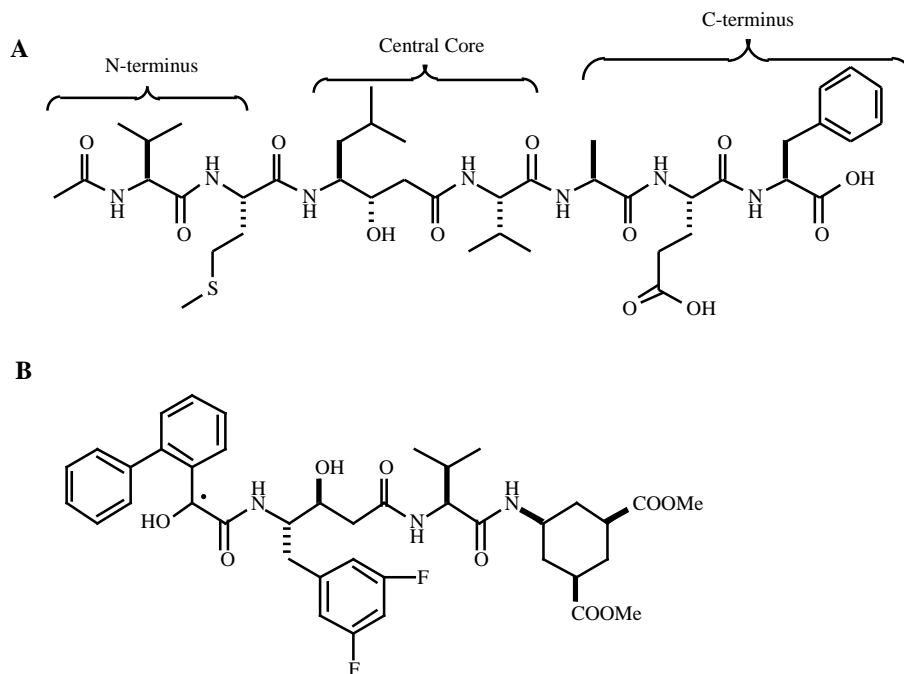


Fig. (5). (A) Schematic representation of the search for improved BACE₁ inhibitors by the reiterative change of N- and C-terminus fragments of a peptide framework leaving intact a central core (reproduced with permission from Hom *et al.*, 2003).

(B). Compounds found to display selective BACE₁ inhibition at low concentrations (reproduced with permission from Hom *et al.*, 2003).

NH₂, which has a low tendency to form the β -pleated sheet conformation. Vaccination with this analogue lowered amyloid burden in the CNS of Tg2576 mice by over 80% [132]. McLaurin and collaborators investigated the differential antibody responses of anti-amyloid antibodies generated by immunizing TgCRND₈ and non-transgenic mice with protofibrillar A β aggregates or the amyloidogenic peptide islet-associated polypeptide (IAPP) not present in the CNS [133]. They concluded that antibodies against the 4-10 A β epitope were effective in inhibiting fibrillogenesis, while they were ineffective in recruiting a pro-inflammatory TH1 response [133], thus suggesting new avenues for an effective immune approach in AD. These epitopes are similar to those originally reported by Solomon *et al.* which effectively blocked fibrillogenesis *in vitro* (see above and [124]). In addition, a recent study found that a significantly higher proportion of healthy elderly subjects and patients with AD had a strong A β -reactive T-cell response than middle aged adults [134]. Further analysis confirmed that the immuno-dominant A β epitopes in humans resided in amino acids 16-33 [134], which would agree with McLaurin *et al.* [133].

Another proposed method of A β clearance [135] is the peripheral sink theory, which stems from the observation that PDAPP mice passively immunized with the Mab m266 (anti-A β ₁₃₋₂₈) displayed a rapid 1000 fold increase in plasma A β levels [136]. The authors propose that the increased plasma A β level resulted from an imbalance in A β concentrations within the CNS and the periphery, which would appear plausible given that A β deposits within the immunized PDAPP brains were in no way labeled by m266 [136]. A subsequent study demonstrated that m266 administration

significantly reduced the age-dependent object recognition memory impairments previously demonstrated in this line [137,138], in a dose dependent manner [139]. Thus, administration of the monoclonal antibody m266 markedly improved learning and memory performance in a dose-dependent manner, without crossing the BBB or altering the hippocampal or cortical amyloid burden. Passive immunization has also been shown to be experimentally effective in removing the CNS A β burden, when anti-A β antibodies are applied intracranially [140]. These experiments also demonstrate a rapid (hours) clearance of diffuse A β material and a delayed (days) clearance of aggregated A β [140]. An overview of the different effects of immunization is shown in (Fig. 6).

Clinical Findings

As previously mentioned the first anti-A β therapeutic to enter clinical trial was Elan Pharmaceutical's AN-1792 vaccine. After completion of Phase I and II clinical trials, the drug entered into a second Phase II clinical trial, involving 375 patients with mild to moderate AD who received either AN-1792 or a placebo drug in a 4:1 ratio [135]. Of the 300 patients receiving AN-1792, 17 reported signs and symptoms consistent with meningoencephalitis (transient changes in MRI scans, elevated white blood cell counts in the CSF etc.), causing the trial to be prematurely halted in January of 2002 [135]. Several patients displayed signs of inflammation prior to the detection of anti-A β antibodies, which suggested that the meningoencephalitis was perhaps T-cell mediated [141]. A case report was recently published which discussed the neuropathology of a deceased participant (pulmonary embo-

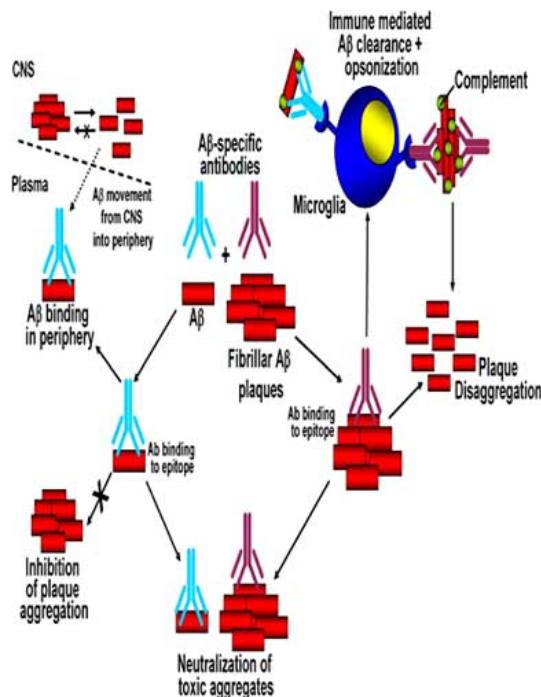


Fig. (6). Diagrammatic representation of the possible modes of action of anti-A β antibodies, in the prevention or breakdown of A β aggregation/fibrillization.

lism) of the Phase II clinical trial [142], as compared to the brains of 7 other non-immunized deceased AD patients. These comparisons revealed several unusual features in the immunized brain: 1) extensive areas of the neocortex contained very few plaques; 2) those cortical plaque absent areas displayed evidence of other neuropathological features associated with AD, such as neurofibrillary tangles and cerebral amyloid angiopathy, but lacked dystrophic neurites and astrocyte clusters; 3) A β in plaque absent areas was associated with microglia; 4) evidence of T-lymphocyte meningoencephalitis was present; and 5) macrophage infiltration was visible in the cerebral white matter [142]. These findings provide further evidence that, despite the apparently reduced amyloid plaque burden, AN-1792 immunization likely initiated an unwanted pro-inflammatory T-cell mediated response, which would explain the observed cases of meningoencephalitis.

An investigation of the antibody production in 30 of the actively immunized AN-1792 participants, showed the presence of A β -specific antibodies in both the plasma and CSF of actively immunized patients, as revealed by immunostaining in AD and transgenic mouse brain tissue [143]. No staining was detectable for full length APP, nor its physiological derivatives, including soluble A β [143]. These findings are extremely relevant, as they provide clear evidence that active immunization with AN-1792 successfully induced the generation of A β -specific antibodies and furthermore, that these antibodies do not cross react with endogenous APP, thereby eliminating the possibility that cross reactivity with normal tissue could have been responsible for the observed cases of meningoencephalitis, as has been suggested [141]. Some investigators, in particular McGeer and McGeer, remain skeptical of the therapeutic value of this approach, as they predict that immunization procedures would likely worsen the inflammatory component of the disease [144].

PREVENTION OF INFLAMMATION

There is an abundant amount of evidence which demonstrates the presence of an inflammatory response in AD neuropathology, an event which was brought to light by the early work of McGeer [145] and Eikelenboom and coworkers [146,147]. We now know that this includes increased levels of colony stimulating factor, C1q [148] and PGE $_2$ [149,150], phagocytic engulfment of A β by microglia [151,152], astrocytic activation [153], proteoglycan deposition surrounding neuritic plaques [154,155] and elevated COX expression [156]. Furthermore, a recent study found that hAPP/sCrry transgenic mice, expressing both A β and a soluble complement receptor-related protein (sCrry) inhibiting C3 (a key component of the complement pathway), displayed a 2 to 3 fold higher deposition of A β , than age-matched hAPP mice [157].

Inflammation is an innate immune response, which facilitates the elimination of xenobiotics, noxious substances and damaged tissue. However, an inflammatory response is not necessarily always beneficial, as it can initiate the generation of free radicals, reactive oxygen and nitrogen species, as well as lipid peroxidation. McGeer, Eikelenboom and Rogers were the earliest researchers to clearly identify the

inflammatory involvement in AD [33,145,146]. This observation was supported by retrospective studies, which found that chronic NSAID users display a lower risk of developing AD than non-NSAID users (for review see [32,33,145,158-160]). The protective effect of chronic NSAID administration was initially attributed to an anti-inflammatory mechanistic action, via the inhibition of the COX 1 and 2 pathways. However, prospective clinical trials involving the NSAID's Diclofenac [161], Celecoxib [162], Prednisone [163] and Naproxen [164,165], all failed to produce any sign of improvement in the participants (for reviews see [166-168]). While disappointing, the results were invaluable as they initiated a number of studies which investigated the COX-independent actions of NSAIDs on various aspects of the AD pathology.

NSAIDs are administered in a racemic mixture of R- and S-enantiomers. Within the profen family, it is the S-enantiomer which elicits the COX inhibitory action [169]. A study by Koo and collaborators, found that the application of ibuprofen, indomethacin and sulindac sulphide preferentially decreased the production of A $_{1-42}$ by as much as 80% in a variety of cultured cells [170]. The study also demonstrated that short-term administration of ibuprofen to Tg2576 mice effectively lowered A $_{1-42}$ levels in the brain, and furthermore that this occurred in a COX independent manner [170], implying the involvement of another pathway or an inherent activity in the R-enantiomer. A subsequent study by Morihara *et al.* [171] found that two supposedly inactive R-enantiomers, R-ibuprofen and R-flurbiprofen, both of which exhibit poor COX inhibition, effectively reduced A $_{1-42}$ production in human cells *in vitro*. Because NSAIDs also mediate the activity of NF κ B, a transcription factor which has been shown to specifically induce A $_{1-42}$ production *in vitro* [172], Morihara *et al.*, [171] repeated the experiment in the presence of a known NF κ B inhibitor, SN-50. Inhibition of the NF κ B pathway had no visible effect on A β production, thus demonstrating that A β production is not mediated by NF κ B inhibition [171]. A recent study by Koo and Golde found that flurbiprofen enantiomers effectively lower A $_{1-42}$ levels in broken cell β -secretase assays, demonstrating that the compounds selectively reduce A $_{1-42}$ production by directly targeting the enzyme complex which cleaves A β from APP ([173] as further discussed in β -secretase section).

Following the initial study by Koo and collaborators [170], one of the original retrospective studies investigating the incidence of AD in NSAID users [174] was re-analyzed and the data presented at the 8th International Congress on AD [175]. The study separated NSAIDs with the ability to lower A $_{1-42}$ production from those which did not, and interestingly found that the reduced incidence of AD was only associated with those NSAIDs capable of reducing A $_{1-42}$ production [175]. In contrast, John Breitner and collaborators performed a longitudinal study termed the "Cache County" study and found that both naproxen and aspirin, neither of which have the ability to lower A $_{1-42}$ levels, elicited a protective effect against the development of AD [176]. Breitner suggested that other clinical trials showed poor results due to the very advanced stages of AD, implying that NSAIDs may only be protective if administered prophylactically. Based on this, Breitner is currently leading a trial, funded by the National Institute on Aging, which will inves-

tigate the long term effect of the twice daily administration of naproxen or celecoxib, over a 5-7 year period, the results of which are expected in 2009 (for discussion see [177]). A recent study by Tarkowski *et al.* provides further evidence to support Breitner's view that prophylactic administration of NSAIDs may be critical for disease prevention [178]. The study analyzed the cerebrospinal fluid levels of tau proteins, A β , a marker for neurodegeneration, the pro-inflammatory cytokines IL-1B and TNF and the anti-inflammatory cytokine TGF β in patients with MCI versus age-matched healthy controls [178]. Interestingly, the study found an increased production of TNF and a decreased production of TGF β in patients with MCI, suggesting that a propensity towards inflammation exists in the AD pathology and that this CNS inflammation is an early hallmark of the disease pathogenesis [178].

CONCLUDING REMARKS

Thanks to the work of a host of researchers, a deeper understanding of the molecular aspects of A β -amyloidosis, and a number of avenues to explore a "disease modifying" therapy have recently opened up, something which seemed very distant only a few short years ago. Furthermore, the development of APP transfected cell lines and the establishment of a number of transgenic animal models reproducing features of AD pathology, the extracellular aggregation of A β into plaque-like structures in particular, have allowed us to investigate the proof of concept for many putative therapies and consequently have led to improvements in lead compounds. This review has commented on the most salient features of this saga from the authors' perspective. It is still too early to predict which routes will be the most promising for the development of drugs or therapies designed to prevent, arrest or reverse the AD pathology along with concomitant cognitive deficits. A substantive problem lies in the fact that once clinical signs of AD become evident and subsequent therapy is initiated, much of the neuronal damage has already been done, since millions and millions of synapses have been lost as well as a good number of neurons, leaving a brain which is burdened by aggregated A β and insoluble neurofibrillary tangles. The realization that Mild Cognitive Impairment might be prodromic of full fledged Alzheimer's disease opens up the possibility of early therapeutic interventions, as would also be the case for individuals bearing familial mutations in the APP, PS1 or PS2 genes. A number of factors, however, continue to be a hindrance to the development and clinical validation of new drugs: 1) the fact that no consensus exists as to which biochemical markers are adequately representative of disease evolution; 2) the lack of accessible and reliable brain imaging methods in order to monitor brain pathology; and 3) assuming 1 and 2 can be overcome, the enormous cost of launching multi-year clinical trials to demonstrate truly "disease modifying" agents, a task which is daunting even for large pharmaceutical enterprises. Nevertheless, the relentless pace and enormous scientific progress made thus far, with respect to potential therapies discussed here, as well as others not mentioned, provide sufficient optimism to believe that a long term control of the AD pathology may eventually be attainable.

ACKNOWLEDGEMENT

This work was supported by Canadian Institutes of Health Research grant # MOP-37996 to Dr. A.C. Cuello. K.F.S. Bell is a recipient of a CIHR Doctoral Research Award.

REFERENCES

- [1] Selkoe, D. J. *Curr. Opin. Neurobiol.* **1994**, *4*(5), 708-716.
- [2] Selkoe, D. J. *Ann. N. Y. Acad. Sci. USA* **2000**, *924*, 17-25.
- [3] Mandelkow, E. M.; Biernat, J.; Drewes, G.; Gustke, N.; Trinczek, B.; Mandelkow, E. *Neurobiology of Aging* **1995**, *16*(3), 355-362.
- [4] Mandelkow, E. M.; Schweers, O.; Drewes, G.; Biernat, J.; Gustke, N.; Trinczek, B.; Mandelkow, E. *Ann. N. Y. Acad. Sci. USA* **1996**, *777*, 96-106.
- [5] Mandelkow, E. M.; Mandelkow, E. *Trends Cell Biol.* **1998**, *8*(11), 425-427.
- [6] Avila, j.; Lim, F.; Moreno, F.; Belmonte, C.; Cuello, A. C. *Mol. Neurobiol.* **2002**, *25*(3), 213-231.
- [7] Younkin, S. G. *J. Physiol. Paris* **1998**, *92*(3-4), 289-292.
- [8] Suzuki, N.; Cheung, T. T.; Cai, X. D.; Odaka, A.; Otvos, L. Jr.; Eckman, C.; Golde, T. E.; Younkin, S. G. *Science* **1994**, *264*(5163), 1336-1340.
- [9] Iqbal, K.; Alonso, A. D.; Gondal, J. A.; Gong, C. X.; Haque, N.; Khatoon, S.; Sengupta, A.; Wang, J. Z.; Grundke-Iqbal, I. *J. Neural Transm. Suppl.* **2000**, *59*, 213-222.
- [10] Iqbal, K.; Alonso, A. C.; El Akkad, E.; Gong, C. X.; Haque, N.; Khatoon, S.; Pei, J. J.; Tsujio, I.; Wang, J. Z.; Grundke-Iqbal, I. *J. Mol. Neurosci.* **2002**, *19*(1-2), 95-99.
- [11] Iqbal, K.; Alonso, A. C.; El Akkad, E.; Gong, C. X.; Haque, N.; Khatoon, S.; Tsujio, I.; Grundke-Iqbal, I. *J. Neural Transm. Suppl.* **2002**, *(62)*, 309-319.
- [12] Lee, V. M.; Goedert, M.; Trojanowski, J. Q. *Annu. Rev. Neurosci.* **2001**, *24*, 1121-1159.
- [13] Goedert, M.; Spillantini, M. G. *Biochim. Biophys. Acta* **2000**, *1502*(1), 110-121.
- [14] Goedert, M.; Spillantini, M. G. *Biochem. Soc. Symp.* **2001**, *(67)*, 59-71.
- [15] Hardy, J. *Trends in Neurosciences* **1997**, *20*(4), 154-159.
- [16] Selkoe, D. J. *Nature* **1999**, *399*(6738 Suppl), A23-A31.
- [17] Dyrks, T.; Weidemann, A.; Multhaup, G.; Salbaum, J. M.; LeMaire, H. G.; Kang, J.; Muller-Hill, B.; Masters, C. L.; Beyreuther, K. *EMBO J.* **1988**, *7*(4), 949-957.
- [18] Citron, M. J. *Neurosci. Res.* **2002**, *70*(3), 373-379.
- [19] Wolfe, M. S.; Haass, C. *J. Biol. Chem.* **2001**, *276*(8), 5413-5416.
- [20] Haass, C.; De Strooper, B. *Science* **1999**, *286*(5441), 916-919.
- [21] Walter, J.; Kaether, C.; Steiner, H.; Haass, C. *Curr. Opin. Neurobiol.* **2001**, *11*(5), 585-590.
- [22] Hardy, J.; Selkoe, D. J. *Science* **2002**, *297*(5580), 353-356.
- [23] Howlett, D. R.; Allsop, D.; Karran, E. H. In *Neurobiology of Alzheimer's disease*, 2 ed.; Dawnbarn, D.; Allen, S. J., editors; Oxford University Press: Oxford, **2001**, Chapter 4, pp. 75-102.
- [24] Terry, R. D.; Masliah, E.; Salmon, D. P.; Butters, N.; DeTeresa, R.; Hill, R.; Hansen, L. A.; Katzman, R. *Annals of Neurology* **1991**, *30*(4), 572-580.
- [25] Terry, R. D. *Journal of Neuropathology and Experimental Neurology* **1996**, *55*(10), 1023-1025.
- [26] Terry, R. D. *Annals of Neurology* **1997**, *41*(1), 7.
- [27] Boncristiano, S.; Calhoun, M. E.; Kelly, P. H.; Pfeifer, M.; Bon-dolfi, L.; Stalder, M.; Phinney, A. L.; Abramowski, D.; Sturchler-Pierrat, C.; Enz, A.; Sommer, B.; Staufenbiel, M.; Jucker, M. *Journal of Neuroscience* **2002**, *22*(8), 3234-3243.
- [28] Cuello, A. C.; Bell, K. F. S.; Echeverria, V.; Lopez, E.; Ribeiro-da-Silva, A.; Szyf, M. The impact of extracellular A β peptides on cortical neurotransmitters and of intracellular Ab accumulation on protein expression, in *Progress in Alzheimer's and Parkinson's Disease Research*, Hanin, I.; Fisher, A.; Cacabelos, R., editors; Martin Dunitz: Chapter, In Press.
- [29] Bell, K. F.; deKort, G. J.; Steggerda, S.; Shigemoto, R.; Ribeiro-da-Silva, A.; Cuello, A. C. *Neurosci. Lett.* **2003**, *353*(2), 143-147.
- [30] Wong, T. P.; Debeir, T.; Duff, K.; Cuello, A. C. *Journal of Neuroscience* **1999**, *19*(7), 2706-2716.
- [31] Hoozemans, J. J.; Veerhuis, R.; Rozemuller, A. J.; Eikelenboom, P. *Drugs Today (Barc.)* **2002**, *38*(6), 429-443.

- [32] McGeer, P. L.; McGeer, E. G. *J. Neurovirol.* **2002**, *8*(6), 529-538.
- [33] Rogers, J.; Webster, S.; Lue, L. F.; Brachova, L.; Civin, W. H.; Emmerling, M.; Shivers, B.; Walker, D.; McGeer, P. *Neurobiology of Aging* **1996**, *17*(5), 681-686.
- [34] Perry, G.; Cash, A. D.; Smith, M. A. *J. Biomed. Biotechnol.* **2002**, *2*(3), 120-123.
- [35] Castegna, A.; Thongboonkerd, V.; Klein, J. B.; Lynn, B.; Markesbery, W. R.; Butterfield, D. A. *J. Neurochem.* **2003**, *85*(6), 1394-1401.
- [36] Mattson, M. P. *Ann. Intern. Med.* **2003**, *139*(5 Pt. 2), 441-444.
- [37] Harper, J. D.; Wong, S. S.; Lieber, C. M.; Lansbury, P. T. Jr. *Biochemistry* **1999**, *38*(28), 8972-8980.
- [38] Howlett, D. R.; Jennings, K. H.; Lee, D. C.; Clark, M. S.; Brown, F.; Wetzel, R.; Wood, S. J.; Camilleri, P.; Roberts, G. W. *Neurodegeneration* **1995**, *4*(1), 23-32.
- [39] Alvarez, G.; Munoz-Montano, J. R.; Satrustegui, J.; Avila, J.; Boggone, E.; Diaz-Nido, J. *Bipolar. Disord.* **2002**, *4*(3), 153-165.
- [40] Kisilevsky, R.; Lemieux, L. J.; Fraser, P. E.; Kong, X.; Hultin, P. G.; Szarek, W. A. *Nat. Med.* **1995**, *1*(2), 143-148.
- [41] Zhu, H.; Yu, J.; Kindy, M. S. *Mol. Med.* **2001**, *7*(8), 517-522.
- [42] Dudas, B.; Cornelli, U.; Lee, J. M.; Hejna, M. J.; Walzer, M.; Lorens, S. A.; Mervis, R. F.; Fareed, J.; Hanin, I. *Neurobiology of Aging* **2002**, *23*(1), 97-104.
- [43] Walzer, M.; Lorens, S.; Hejna, M.; Fareed, J.; Hanin, I.; Cornelli, U.; Lee, J. M. *Eur. J. Pharmacol.* **2002**, *445*(3), 211-220.
- [44] Dudas, B.; Cornelli, U.; Rose, M.; Hanin, I. Low molecular weight glycosaminoglycans protect against AF64A-induced cholinergic lesions in rat brain. Society for Neuroscience abstracts [Program No 483.8]. 1-11-2002.
- [45] Rose, M.; Dudas, B.; Cornelli, U.; Hanin, I. *Neurobiology of Aging* **2003**, *24*(3), 481-490.
- [46] Gervais, F.; Chalifour, R.; Garceau, D.; Kong, X.; Laurin, J.; McLaughlin, R.; Morissette, C.; Paquette, J. *Amyloid* **2001**, *8 Suppl 1* 28-35.
- [47] Soto, C.; Kindy, M. S.; Baumann, M.; Frangione, B. *Biochem. Biophys. Res Commun.* **1996**, *226*(3), 672-680.
- [48] Wouters, M. A.; Curmi, P. M. *Proteins* **1995**, *22*(2), 119-131.
- [49] Wood, S. J.; Wetzel, R.; Martin, J. D.; Hurle, M. R. *Biochemistry* **1995**, *34*(3), 724-730.
- [50] Poduslo, J. F.; Curran, G. L.; Kumar, A.; Frangione, B.; Soto, C. *J. Neurobiol.* **1999**, *39*(3), 371-382.
- [51] Tjernberg, L. O.; Naslund, J.; Lindqvist, F.; Johansson, J.; Karlstrom, A. R.; Thyberg, J.; Terenius, L.; Nordstedt, C. *J. Biol. Chem.* **1996**, *271*(15), 8545-8548.
- [52] Tjernberg, L. O.; Lilliehook, C.; Callaway, D. J.; Naslund, J.; Hahne, S.; Thyberg, J.; Terenius, L.; Nordstedt, C. *J. Biol. Chem.* **1997**, *272*(19), 12601-12605.
- [53] Akikusa, S.; Watanabe, K. I.; Horikawa, E.; Nakamura, K.; Kodaka, M.; Okuno, H.; Konakahara, T. *J. Pept. Res.* **2003**, *61*(1), 1-6.
- [54] Gordon, D. J.; Sciarretta, K. L.; Meredith, S. C. *Biochemistry* **2001**, *40*(28), 8237-8245.
- [55] Hetenyi, C.; Szabo, Z.; Klement, E.; Datki, Z.; Kortvelyesi, T.; Zarandi, M.; Penke, B. *Biochem. Biophys. Res. Commun.* **2002**, *292*(4), 931-936.
- [56] Chalifour, R. J.; McLaughlin, R. W.; Lavoie, L.; Morissette, C.; Tremblay, N.; Boule, M.; Sarazin, P.; Stea, D.; Lacombe, D.; Tremblay, P.; Gervais, F. *J. Biol. Chem.* **2003**, *278*(37), 34874-81.
- [57] Lorenzo, A.; Yankner, B. A. *Proc. Natl. Acad. Sci. USA* **1994**, *91*(25), 12243-12247.
- [58] Podlisny, M. B.; Walsh, D. M.; Amarante, P.; Ostaszewski, B. L.; Stimson, E. R.; Maggio, J. E.; Teplow, D. B.; Selkoe, D. J. *Biochemistry* **1998**, *37*(11), 3602-3611.
- [59] Klunk, W. E.; Debnath, M. L.; Pettegrew, J. W. *Neurobiology of Aging* **1994**, *15*(6), 691-698.
- [60] Klunk, W. E.; Debnath, M. L.; Koros, A. M.; Pettegrew, J. W. *Life Sci.* **1998**, *63*(20), 1807-1814.
- [61] Bush, A. I.; Pettingell, W. H. Jr.; Paradis, M. D.; Tanzi, R. E. *J. Biol. Chem.* **1994**, *269*(16), 12152-12158.
- [62] Bush, A. I.; Pettingell, W. H.; Multhaup, G.; Paradis, M.; Vonsattel, J. P.; Gusella, J. F.; Beyreuther, K.; Masters, C. L.; Tanzi, R. E. *Science* **1994**, *265*(5177), 1464-1467.
- [63] Miura, T.; Suzuki, K.; Kohata, N.; Takeuchi, H. *Biochemistry* **2000**, *39*(23), 7024-7031.
- [64] Tabira, T. *Science* **2001**, *292*(5525), 2251-2252.
- [65] Cherny, R. A.; Atwood, C. S.; Xilinas, M. E.; Gray, D. N.; Jones, W. D.; McLean, C. A.; Barnham, K. J.; Volitakis, I.; Fraser, F. W.; Kim, Y.; Huang, X.; Goldstein, L. E.; Moir, R. D.; Lim, J. T.; Beyreuther, K.; Zheng, H.; Tanzi, R. E.; Masters, C. L.; Bush, A. I. *Neuron* **2001**, *30*(3), 665-676.
- [66] Regland, B.; Lehmann, W.; Abedini, I.; Blennow, K.; Jonsson, M.; Karlsson, I.; Sjogren, M.; Wallin, A.; Xilinas, M.; Gottfries, C. G. *Dement. Geriatr. Cogn. Disord.* **2001**, *12*(6), 408-414.
- [67] Finebrock, A. E.; Bush, A. I.; Doraiswamy, P. M. *J. Am. Geriatr. Soc.* **2003**, *51*(8), 1143-1148.
- [68] Tomiyama, T.; Asano, S.; Suwa, Y.; Morita, T.; Kataoka, K.; Mori, H.; Endo, N. *Biochem. Biophys. Res Commun.* **1994**, *204*(1), 76-83.
- [69] Tomiyama, T.; Kaneko, H.; Kataoka, K.; Asano, S.; Endo, N. *Biochem. J.* **1997**, *322* (Pt. 3) 859-865.
- [70] Savage, M. J.; Trusko, S. P.; Howland, D. S.; Pinsker, L. R.; Mistretta, S.; Reaume, A. G.; Greenberg, B. D.; Siman, R.; Scott, R. W. *Journal of Neuroscience* **1998**, *18*(5), 1743-1752.
- [71] LeBlanc, A. C.; Koutroumanis, M.; Goodyer, C. G. *Journal of Neuroscience* **1998**, *18*(8), 2907-2913.
- [72] Hooper, N. M.; Turner, A. J. *Curr. Med. Chem.* **2002**, *9*(11), 1107-1119.
- [73] Kozikowski, A. P.; Nowak, I.; Petukhov, P. A.; Etcheberrigaray, R.; Mohamed, A.; Tan, M.; Lewin, N.; Hennings, H.; Pearce, L. L.; Blumberg, P. M. *J. Med. Chem.* **2003**, *46*(3), 364-373.
- [74] Bowen, D. M.; Smith, C. D. *Brain* **1976**, *99*, 459-496.
- [75] Davies, P.; Maloney, A. J. F. *Lancet* **1976**, *2* (8000), 1403.
- [76] Whitehouse, P. J.; Price, D. L.; Struble, R. G.; Clark, A. W.; Coyle, J. T.; DeLong, M. R. *Science* **1982**, *215*, 1237-1239.
- [77] Cuello, A. C.; Sofroniew, M. V. *Trends in Neurosciences* **1984**, *7* 74-78.
- [78] Pearson, R. C.; Sofroniew, M. V.; Cuello, A. C.; Powell, T. P.; Eckenstein, F.; Esiri, M. M.; Wilcock, G. K. *Brain Res.* **1983**, *289*(1-2), 375-379.
- [79] Nitsch, R. M.; Slack, B. E.; Wurtman, R. J.; Growdon, J. H. *Science* **1992**, *258*(5080), 304-307.
- [80] Nitsch, R. M.; Farber, S. A.; Growdon, J. H.; Wurtman, R. J. *Proc. Natl. Acad. Sci. USA* **1993**, *90*(11), 5191-5193.
- [81] Mori, F.; Lai, C. C.; Fusi, F.; Giacobini, E. *Neuroreport* **1995**, *6*(4), 633-636.
- [82] Beach, T. G.; Walker, D. G.; Potter, P. E.; Sue, L. I.; Fisher, A. *Brain Res.* **2001**, *905*(1-2), 220-223.
- [83] Olson, L.; Nordberg, A.; von Holst, H.; Backman, L.; Ebendal, T.; Alafuzoff, I.; Amberla, K.; Hartvig, P.; Herlitz, A.; Lilja, A.; J. *Neural Transm. Park Dis. Dement. Sect.* **1992**, *4*(1), 79-95.
- [84] Cuello, A. C.; Thoenen, H. In *Pharmacological Sciences: Perspectives for Research and Therapy in the Late 1990s*, 1 ed.; Cuello, A. C.; Collier, B.; editors; Birkhauser: Basel, **1995**, pp. 241-254.
- [85] Garofalo, L.; Ribeiro-da-Silva, A.; Cuello, A. C. *Proc. Natl. Acad. Sci. USA* **1992**, *89*(7), 2639-2643.
- [86] Fischer, W.; Victorin, K.; Bjorklund, A.; Williams, L. R.; Varon, S.; Gage, F. H. *Nature* **1987**, *329*(6134), 65-68.
- [87] Bruno, M.A., Clarke, P.B.; Seltzer, A.; Quirion, R.; Burgess, K.; Cuello, A.C.; Saragovi, H.U. *J. Neurosci.* **2004**, *24*(37), 8009-8018.
- [88] De Strooper, B. *Neuron* **2003**, *38*(1), 9-12.
- [89] Wolfe, M. S. *Int. Immunopharmacol.* **2002**, *2*(13-14), 1919-1929.
- [90] Hartmann, D.; Tournoy, J.; Saftig, P.; Annaert, W.; De Strooper, B. *J. Mol. Neurosci.* **2001**, *17*(2), 171-181.
- [91] Wolfe, M. S.; De Los, A. J.; Miller, D. D.; Xia, W.; Selkoe, D. J. *Biochemistry* **1999**, *38*(35), 11223-11230.
- [92] Li, Y. M.; Xu, M.; Lai, M. T.; Huang, Q.; Castro, J. L.; DiMuzio-Mower, J.; Harrison, T.; Lellis, C.; Nadin, A.; Neduveilil, J. G.; Register, R. B.; Sardana, M. K.; Shearman, M. S.; Smith, A. L.; Shi, X. P.; Yin, K. C.; Shafer, J. A.; Gardell, S. J. *Nature* **2000**, *405*(6787), 689-694.
- [93] Maiorini, A. F.; Gaunt, M. J.; Jacobsen, T. M.; McKay, A. E.; Waldman, L. D.; Raffa, R. B. *J. Clin. Pharm. Ther.* **2002**, *27*(3), 169-183.
- [94] Dovey, H. F.; John, V.; Anderson, J. P.; Chen, L. Z.; de Saint, A. P.; Fang, L. Y.; Freedman, S. B.; Folmer, B.; Goldberg, E.; Holtzman, E. J.; Hu, K. L.; Johnson-Wood, K. L.; Kennedy, S. L.; Kholodenko, D.; Knops, J. E.; Latimer, L. H.; Lee, M.; Liao, Z.; Lieberburg, I. M.; Motter, R. N.; Mutter, L. C.; Nietz, J.; Quinn, K. P.; Sacchi, K. L.; Seubert, P. A.; Shopp, G. M.; Thorsett, E. D.; Tung, J. S.; Wu, J.; Yang, S.; Yin, C. T.; Schenk, D. B.; May, P.

- C.; Altstiel, L. D.; Bender, M. H.; Boggs, L. N.; Britton, T. C.; Clemens, J. C.; Czilli, D. L.; Dieckman-McGinty, D. K.; Droste, J. J.; Fuson, K. S.; Gitter, B. D.; Hyslop, P. A.; Johnstone, E. M.; Li, W. Y.; Little, S. P.; Mabry, T. E.; Miller, F. D.; Audia, J. E. *J. Neurochem.* **2001**, *76*(1), 173-181.
- [95] Games, D.; Adams, D.; Alessandrini, R.; Barbour, R.; Berthelette, P.; Blackwell, C.; Carr, T.; Clemens, J.; Donaldson, T.; Gillespie, F.; Guido, T.; Hagopian, S.; Johnson-Wood, K.; Khan, K.; Lee, M.; Leibowitz, P.; Lieberburg, I.; Little, S.; Masliah, E.; McConlogue, L.; Montoya-Zavala, M.; Mucke, L.; Paganini, L.; Penniman, E.; Power, M.; Schenk, D.; Seubert, P.; Snyder, B.; Soriano, F.; Tan, H.; Vitale, J.; Wadsworth, S.; Wolozin, B.; Zhao, J. *Nature* **1995**, *373*(6514), 523-527.
- [96] Lanz, T. A.; Himes, C. S.; Pallante, G.; Adams, L.; Yamazaki, S.; Amore, B.; Merchant, K. M. *J. Pharmacol. Exp. Ther.* **2003**, *305*(3), 864-871.
- [97] Hsiao, K.; Chapman, P.; Nilsen, S.; Eckman, C.; Harigaya, Y.; Younkin, S.; Yang, F.; Cole, G. *Science* **1996**, *274*(5284), 99-102.
- [98] Shen, J.; Bronson, R. T.; Chen, D. F.; Xia, W.; Selkoe, D. J.; Tonegawa, S. *Cell* **1997**, *89*(4), 629-639.
- [99] Ni, C. Y.; Murphy, M. P.; Golde, T. E.; Carpenter, G. *Science* **2001**, *294*(5549), 2179-2181.
- [100] Leissring, M. A.; Murphy, M. P.; Mead, T. R.; Akbari, Y.; Sugarman, M. C.; Jannatipour, M.; Anliker, B.; Muller, U.; Saftig, P.; De Strooper, B.; Wolfe, M. S.; Golde, T. E.; LaFerla, F. M. *Proc. Natl. Acad. Sci. USA* **2002**, *99*(7), 4697-4702.
- [101] Lewis, H. D.; Perez Revuelta, B. I.; Nadin, A.; Neduvilil, J. G.; Harrison, T.; Pollack, S. J.; Shearman, M. S. *Biochemistry* **2003**, *42*(24), 7580-7586.
- [102] Kornilova, A. Y.; Das, C.; Wolfe, M. S. *J. Biol. Chem.* **2003**, *278*(19), 16470-16473.
- [103] Petit, A.; Bihel, F.; Alves, d. C.; Pourquie, O.; Checler, F.; Kraus, J. L. *Nat. Cell Biol.* **2001**, *3*(5), 507-511.
- [104] Esler, W. P.; Das, C.; Campbell, W. A.; Kimberly, W. T.; Kornilova, A. Y.; Diehl, T. S.; Ye, W.; Ostaszewski, B. L.; Xia, W.; Selkoe, D. J.; Wolfe, M. S. *Nat. Cell Biol.* **2002**, *4*(5), E110-E111.
- [105] Tian, G.; Sobotka-Briner, C. D.; Zysk, J.; Liu, X.; Birr, C.; Sylvester, M. A.; Edwards, P. D.; Scott, C. D.; Greenberg, B. D. *J. Biol. Chem.* **2002**, *277*(35), 31499-31505.
- [106] Tian, G.; Ghanekar, S. V.; Aharony, D.; Shenvi, A. B.; Jacobs, R. T.; Liu, X.; Greenberg, B. D. *J. Biol. Chem.* **2003**, *278*(31), 28968-28975.
- [107] Phiel, C. J.; Wilson, C. A.; Lee, V. M.; Klein, P. S. *Nature* **2003**, *423*(6938), 435-439.
- [108] Haass, C.; Lemere, C. A.; Capell, A.; Citron, M.; Seubert, P.; Schenk, D.; Lannfelt, L.; Selkoe, D. J. *Nat. Med.* **1995**, *1*(12), 1291-1296.
- [109] Vassar, R.; Bennett, B. D.; Babu-Khan, S.; Kahn, S.; Mendiaz, E. A.; Denis, P.; Teplow, D. B.; Ross, S.; Amarante, P.; Loeloff, R.; Luo, Y.; Fisher, S.; Fuller, J.; Edenson, S.; Lile, J.; Jarosinski, M. A.; Biere, A. L.; Curran, E.; Burgess, T.; Louis, J. C.; Collins, F.; Treanor, J.; Rogers, G.; Citron, M. *Science* **1999**, *286*(5440), 735-741.
- [110] Saunders, A. J.; Kim, T.; Tanzi, R. BACE maps to Chromosome 11 and a BACE homolog, BACE 2, Reside in the Obligate Down Syndrome Region of Chromosome 21. *Science* **1999**, *286*(5443), 1255.
- [111] Citron, M. *Neurobiology of Aging* **2002**, *23*(6), 1017-1022.
- [112] Luo, Y.; Bolon, B.; Kahn, S.; Bennett, B. D.; Babu-Khan, S.; Denis, P.; Fan, W.; Kha, H.; Zhang, J.; Gong, Y.; Martin, L.; Louis, J. C.; Yan, Q.; Richards, W. G.; Citron, M.; Vassar, R. *Nat. Neurosci.* **2001**, *4*(3), 231-232.
- [113] Roberds, S. L.; Anderson, J.; Basi, G.; Bienkowski, M. J.; Branstetter, D. G.; Chen, K. S.; Freedman, S. B.; Frigon, N. L.; Games, D.; Hu, K.; Johnson-Wood, K.; Kappenman, K. E.; Kawabe, T. T.; Kola, I.; Kuehn, R.; Lee, M.; Liu, W.; Motter, R.; Nichols, N. F.; Power, M.; Robertson, D. W.; Schenk, D.; Schoor, M.; Shopp, G. M.; Shuck, M. E.; Sinha, S.; Svensson, K. A.; Tatsuno, G.; Tintrup, H.; Wijsman, J.; Wright, S.; McConlogue, L. *Hum. Mol. Genet.* **2001**, *10*(12), 1317-1324.
- [114] Fischer, F.; Molinari, M.; Bodendorf, U.; Paganetti, P. *J. Neurochem.* **2002**, *80*(6), 1079-1088.
- [115] Farzan, M.; Schnitzler, C. E.; Vasilieva, N.; Leung, D.; Choe, H. *Proc. Natl. Acad. Sci. USA* **2000**, *97*(17), 9712-9717.
- [116] Bennett, B. D.; Babu-Khan, S.; Loeloff, R.; Louis, J. C.; Curran, E.; Citron, M.; Vassar, R. *J. Biol. Chem.* **2000**, *275*(27), 20647-20651.
- [117] Hussain, I.; Powell, D. J.; Howlett, D. R.; Chapman, G. A.; Gilmour, L.; Murdock, P. R.; Tew, D. G.; Meek, T. D.; Chapman, C.; Schneider, K.; Ratcliffe, S. J.; Tattersall, D.; Testa, T. T.; Southan, C.; Ryan, D. M.; Simmons, D. L.; Walsh, F. S.; Dingwall, C.; Christie, G. *Mol. Cell Neurosci.* **2000**, *16*(5), 609-619.
- [118] Hong, L.; Koelsch, G.; Lin, X.; Wu, S.; Terzyan, S.; Ghosh, A. K.; Zhang, X. C.; Tang, J. *Science* **2000**, *290*(5489), 150-153.
- [119] Ghosh, A. K.; Bilcer, G.; Harwood, C.; Kawahama, R.; Shin, D.; Hussain, K. A.; Hong, L.; Loy, J. A.; Nguyen, C.; Koelsch, G.; Ermolieff, J.; Tang, J. *J. Med. Chem.* **2001**, *44*(18), 2865-2868.
- [120] Turner, R. T., III; Koelsch, G.; Hong, L.; Castanheira, P.; Ermolieff, J.; Ghosh, A. K.; Tang, J.; Castanheira, P.; Ghosh, A. *Biochemistry* **2001**, *40*(34), 10001-10006.
- [121] Tung, J. S.; Davis, D. L.; Anderson, J. P.; Walker, D. E.; Mamo, S.; Jewett, N.; Hom, R. K.; Sinha, S.; Thorsett, E. D.; John, V. *J. Med. Chem.* **2002**, *45*(2), 259-262.
- [122] Hom, R. K.; Fang, L. Y.; Mamo, S.; Tung, J. S.; Guinn, A. C.; Walker, D. E.; Davis, D. L.; Gailunas, A. F.; Thorsett, E. D.; Sinha, S.; Knops, J. E.; Jewett, N. E.; Anderson, J. P.; John, V. *J. Med. Chem.* **2003**, *46*(10), 1799-1802.
- [123] Solomon, B.; Koppel, R.; Hanan, E.; Katzav, T. *Proc. Natl. Acad. Sci. U. S. A* **1996**, *93*(1), 452-455.
- [124] Solomon, B.; Koppel, R.; Frankel, D.; Hanan-Aharon, E. *Proc. Natl. Acad. Sci. U. S. A* **1997**, *94*(8), 4109-4112.
- [125] Schenk, D.; Barbour, R.; Dunn, W.; Gordon, G.; Grajeda, H.; Guido, T.; Hu, K.; Huang, J.; Johnson-Wood, K.; Khan, K.; Kholodenko, D.; Lee, M.; Liao, Z.; Lieberburg, I.; Motter, R.; Mutter, L.; Soriano, F.; Shopp, G.; Vasquez, N.; Vandeventer, C.; Walker, S.; Wogulis, M.; Yednock, T.; Games, D.; Seubert, P. *Nature* **1999**, *400*(6740), 173-177.
- [126] Bard, F.; Cannon, C.; Barbour, R.; Burke, R. L.; Games, D.; Grajeda, H.; Guido, T.; Hu, K.; Huang, J.; Johnson-Wood, K.; Khan, K.; Kholodenko, D.; Lee, M.; Lieberburg, I.; Motter, R.; Nguyen, M.; Soriano, F.; Vasquez, N.; Weiss, K.; Welch, B.; Seubert, P.; Schenk, D.; Yednock, T. *Nat. Med.* **2000**, *6*(8), 916-919.
- [127] Backskai, B. J.; Kajdasz, S. T.; Christie, R. H.; Carter, C.; Games, D.; Seubert, P.; Schenk, D.; Hyman, B. T. *Nat. Med.* **2001**, *7*(3), 369-372.
- [128] Janus, C.; Pearson, J.; McLaurin, J.; Mathews, P. M.; Jiang, Y.; Schmidt, S. D.; Chishti, M. A.; Horne, P.; Heslin, D.; French, J.; Mount, H. T.; Nixon, R. A.; Mercken, M.; Bergeron, C.; Fraser, P. E.; George-Hyslop, P.; Westaway, D. *Nature* **2000**, *408*(6815), 979-982.
- [129] Morgan, D.; Diamond, D. M.; Gottschall, P. E.; Ugen, K. E.; Dickey, C.; Hardy, J.; Duff, K.; Jantzen, P.; DiCarlo, G.; Wilcock, D.; Connor, K.; Hatcher, J.; Hope, C.; Gordon, M.; Arendash, G. W. *Nature* **2000**, *408*(6815), 982-985.
- [130] Lemere, C. A.; Spooner, E. T.; Leverone, J. F.; Mori, C.; Clements, J. D. *Neurobiology of Aging* **2002**, *23*(6), 991-1000.
- [131] Sigurdsson, E. M.; Wisniewski, T.; Frangione, B. *Neurobiology of Aging* **2002**, *23*(6), 1001-1008.
- [132] Sigurdsson, E. M.; Scholtzova, H.; Mehta, P. D.; Frangione, B.; Wisniewski, T. *Am. J. Pathol.* **2001**, *159*(2), 439-447.
- [133] McLaurin, J.; Cecal, R.; Kierstead, M. E.; Tian, X.; Phinney, A. L.; Manea, M.; French, J. E.; Lamberon, M. H.; Darabie, A. A.; Brown, M. E.; Janus, C.; Chishti, M. A.; Horne, P.; Westaway, D.; Fraser, P. E.; Mount, H. T.; Przybylski, M.; George-Hyslop, P. *Nat. Med.* **2002**, *8*(11), 1263-1269.
- [134] Monsonego, A.; Zota, V.; Karmi, A.; Krieger, J. I.; Bar-Or, A.; Bitan, G.; Budson, A. E.; Sperling, R.; Selkoe, D. J.; Weiner, H. L. *J. Clin. Invest.* **2003**, *112*(3), 415-422.
- [135] Schenk, D. *Nat. Rev. Neurosci.* **2002**, *3*(10), 824-828.
- [136] DeMattos, R. B.; Bales, K. R.; Cummins, D. J.; Dodart, J. C.; Paul, S. M.; Holtzman, D. M. *Proc. Natl. Acad. Sci. USA* **2001**, *98*(15), 8850-8855.
- [137] Dodart, J. C.; Meziane, H.; Mathis, C.; Bales, K. R.; Paul, S. M.; Ungerer, A. *Behav. Neurosci.* **1999**, *113*(5), 982-990.
- [138] Dodart, J. C.; Mathis, C.; Bales, K. R.; Paul, S. M.; Ungerer, A. *Neuroreport* **2000**, *11*(3), 603-607.
- [139] Dodart, J. C.; Bales, K. R.; Gannon, K. S.; Greene, S. J.; DeMattos, R. B.; Mathis, C.; DeLong, C. A.; Wu, S.; Wu, X.; Holtzman, D. M.; Paul, S. M. *Nat. Neurosci.* **2002**, *5*(5), 452-457.

- [140] Wilcock, D. M.; DiCarlo, G.; Henderson, D.; Jackson, J.; Clarke, K.; Ugen, K. E.; Gordon, M. N.; Morgan, D. *Journal of Neuroscience* **2003**, *23*(9), 3745-3751.
- [141] Weiner, H. L.; Selkoe, D. J. *Nature* **2002**, *420*(6917), 879-884.
- [142] Nicoll, J. A.; Wilkinson, D.; Holmes, C.; Steart, P.; Markham, H.; Weller, R. O. *Nat. Med.* **2003**, *9*(4), 448-452.
- [143] Hock, C.; Konietzko, U.; Papassotiropoulos, A.; Wollmer, A.; Streffer, J.; von Rotz, R. C.; Davey, G.; Moritz, E.; Nitsch, R. M. *Nat. Med.* **2002**, *8*(11), 1270-1275.
- [144] McGeer, P. L.; McGeer, E. *Neurobiology of Aging* **2003**, *24*(3), 391-395.
- [145] McGeer, P. L.; Rogers, J.; McGeer, E. G. *Alzheimer Dis. Assoc. Disord.* **1994**, *8*(3), 149-158.
- [146] Eikelenboom, P.; Rozemuller, J. M.; Kraal, G.; Stam, F. C.; McBride, P. A.; Bruce, M. E.; Fraser, H. *Virchows Arch. B Cell Pathol. Incl. Mol Pathol.* **1991**, *60*(5), 329-336.
- [147] Eikelenboom, P.; Zhan, S. S.; van Gool, W. A.; Allsop, D. *Trends Pharmacol. Sci.* **1994**, *15*(12), 447-450.
- [148] Lue, L. F.; Rydel, R.; Brigham, E. F.; Yang, L. B.; Hampel, H.; Murphy, G. M., Jr.; Brachova, L.; Yan, S. D.; Walker, D. G.; Shen, Y.; Rogers, J. *Glia* **2001**, *35*(1), 72-79.
- [149] Bales, K. R.; Du, Y.; Holtzman, D.; Cordell, B.; Paul, S. M. *Neurobiology of Aging* **2000**, *21*(3), 427-432.
- [150] Hu, J.; Van Eldik, L. J. *Brain Res* **1999**, *842*(1), 46-54.
- [151] Frautschy, S. A.; Cole, G. M.; Baird, A. *Am. J. Pathol.* **1992**, *140*(6), 1389-1399.
- [152] Newcomb, R. D.; Jose, R. T. *J. Am. Optom. Assoc.* **1977**, *48*(2), 173-182.
- [153] Nichols, N. R.; Day, J. R.; Laping, N. J.; Johnson, S. A.; Finch, C. E. *Neurobiology of Aging* **1993**, *14*(5), 421-429.
- [154] DeWitt, D. A.; Perry, G.; Cohen, M.; Doller, C.; Silver, J. *Exp. Neurol.* **1998**, *149*(2), 329-340.
- [155] Shaffer, L. M.; Dority, M. D.; Gupta-Bansal, R.; Frederickson, R. C.; Younkin, S. G.; Brunden, K. R. *Neurobiology of Aging* **1995**, *16*(5), 737-745.
- [156] Ho, L.; Purohit, D.; Haroutunian, V.; Luteran, J. D.; Willis, F.; Naslund, J.; Buxbaum, J. D.; Mohs, R. C.; Aisen, P. S.; Pasinetti, G. M. *Arch. Neurol.* **2001**, *58*(3), 487-492.
- [157] Wyss-Coray, T.; Yan, F.; Lin, A. H.; Lambiris, J. D.; Alexander, J. J.; Quigg, R. J.; Masliah, E. *Proc. Natl. Acad. Sci. USA* **2002**, *99*(16), 10837-10842.
- [158] McGeer, P. L.; Schulzer, M.; McGeer, E. G. *Neurology* **1996**, *47*(2), 425-432.
- [159] McGeer, P. L.; McGeer, E. G. *Ann. N. Y. Acad. Sci. USA* **1996**, *777*, 213-220.
- [160] McGeer, P. L.; McGeer, E. G. *Brain Research Reviews* **1995**, *21*(2), 195-218.
- [161] Scharf, S.; Mander, A.; Ugoni, A.; Vajda, F.; Christophidis, N. *Neurology* **1999**, *53*(1), 197-201.
- [162] Sainati, S. M.; Ingram, D. M.; Talwaker, S. Results of a double-blind, placebo-controlled study of celecoxib in the treatment of progression of Alzheimer's disease. Proceedings of the Sixth International Stockholm-Springfield Symposium on Advances in Alzheimer's Therapy **2000**, *180*.
- [163] Aisen, P. S.; Davis, K. L.; Berg, J. D.; Schafer, K.; Campbell, K.; Thomas, R. G.; Weiner, M. F.; Farlow, M. R.; Sano, M.; Grundman, M.; Thal, L. J. *Neurology* **2000**, *54*(3), 588-593.
- [164] Aisen, P. S.; Schafer, K. A.; Grundman, M.; Pfeiffer, E.; Sano, M.; Davis, K. L.; Farlow, M. R.; Jin, S.; Thomas, R. G.; Thal, L. J. *JAMA* **2003**, *289*(21), 2819-2826.
- [165] Tabet, N.; Feldman, H. *Cochrane. Database. Syst. Rev.* **2003**, (2), CD004031.
- [166] Breitner, J. C.; Zandi, P. P. *New England Journal of Medicine* **2001**, *345*(21), 1567-1568.
- [167] Zandi, P. P.; Breitner, J. C. *Neurobiology of Aging* **2001**, *22*(6), 811-817.
- [168] Aisen, P. S. *Lancet Neurol.* **2002**, *1*(5), 279-284.
- [169] Jerussi, T. P.; Caubet, J. F.; McCray, J. E.; Handley, D. A. *J. Clin. Pharmacol.* **1998**, *38*(2 Suppl), 19S-24S.
- [170] Weggen, S.; Eriksen, J. L.; Das, P.; Sagi, S. A.; Wang, R.; Pietrzik, C. U.; Findlay, K. A.; Smith, T. E.; Murphy, M. P.; Bulter, T.; Kang, D. E.; Marquez-Sterling, N.; Golde, T. E.; Koo, E. H. *Nature* **2001**, *414*(6860), 212-216.
- [171] Morihara, T.; Chu, T.; Ubeda, O.; Beech, W.; Cole, G. M. *J. Neurochem.* **2002**, *83*(4), 1009-1012.
- [172] Tomita, S.; Fujita, T.; Kirino, Y.; Suzuki, T. *J. Biol. Chem.* **2000**, *275*(17), 13056-13060.
- [173] Eriksen, J. L.; Sagi, S. A.; Smith, T. E.; Weggen, S.; Das, P.; McLendon, D. C.; Ozols, V. V.; Jessing, K. W.; Zavitz, K. H.; Koo, E. H.; Golde, T. E. *J. Clin. Invest.* **2003**, *112*(3), 440-449.
- [174] in 't Veld, B.; Ruitenber, A.; Hofman, A.; Launer, L. J.; van Duijn, C. M.; Stijnen, T.; Breteler, M. M.; Stricker, B. H. *New Engl. J. Med.* **2001**, *345*(21), 1515-1521.
- [175] Breteler, M. M.; in, t., V.; Hofman, A.; Stricker, B. H. Abeta42 peptide lowering NSAIDs and Alzheimer's disease. 8th International Congress on Alzheimer's Disease Conf ID 02070203. 20-7-2002.
- [176] Zandi, P. P.; Anthony, J. C.; Hayden, K. M.; Mehta, K.; Mayer, L.; Breitner, J. C. *Neurology* **2002**, *59*(6), 880-886.
- [177] Helmuth, L. *Science* **2002**, *297*(5585), 1262-1263.
- [178] Tarkowski, E.; Andreasen, N.; Tarkowski, A.; Blennow, K. *J. Neurol. Neurosurg. Psychiatry* **2003**, *74*(9), 1200-1205.