

## Review Article

# $\beta$ -Amyloid Degradation and Alzheimer's Disease

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Extensive  $\beta$ -amyloid ( $A\beta$ ) deposits in brain parenchyma in the form of senile plaques and in blood vessels in the form of amyloid angiopathy are pathological hallmarks of Alzheimer's disease (AD). The mechanisms underlying  $A\beta$  deposition remain unclear. Major efforts have focused on  $A\beta$  production, but there is little to suggest that increased production of  $A\beta$  plays a role in  $A\beta$  deposition, except for rare familial forms of AD. Thus, other mechanisms must be involved in the accumulation of  $A\beta$  in AD. Recent data shows that impaired clearance may play an important role in  $A\beta$  accumulation in the pathogenesis of AD. This review focuses on our current knowledge of  $A\beta$ -degrading enzymes, including neprilysin (NEP), endothelin-converting enzyme (ECE), insulin-degrading enzyme (IDE), angiotensin-converting enzyme (ACE), and the plasmin/uPA/tPA system as they relate to amyloid deposition in AD.

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## INTRODUCTION

Alzheimer's disease (AD) is the commonest cause of senile dementia and increases in frequency with age. Clinically, AD is characterized by early and progressive memory loss due to neuronal and synaptic loss in the cortex and limbic structures, including the hippocampus and amygdala. In later stages of the disease process, the extensive involvement of cortical and subcortical regions results in loss of higher cognitive abilities, including speech and praxis, and in impaired motor abilities. Grossly, AD brains show global atrophy and reduced weight and volume. Histologically, AD is characterized by amyloid plaques, neurofibrillary tangles, dystrophic neurites, extensive neuronal loss, and gliosis.

Although beta-amyloid ( $A\beta$ ) accumulation and senile/neuritic plaque formation are striking morphological hallmarks of AD and widely used in the neuropathologic diagnosis of AD, it is clearly recognized that amyloid deposition in the brain parenchyma and in vessels also is common for nondemented individuals in advanced age. Many possible explanations for excessive  $A\beta$  deposition have been put forward, including increased production, decreased degradation, and abnormal transport between brain parenchyma and plasma or CSF [1–3]. Although overproduction of  $A\beta$  is critical to the pathogenesis of some forms of familial AD, there is still little evidence to suggest that increased  $A\beta$  production is

important in amyloid deposition in aging and sporadic AD. Recently, the role of degradation has been increasingly recognized in  $A\beta$  homeostasis. Several enzymes have been described with a range of abilities to degrade  $A\beta$ . This review will focus on enzymes capable of degrading  $A\beta$  and their potential significance to the pathogenesis of AD.

## THE AMYLOID CASCADE HYPOTHESIS

The mechanisms underlying the pathogenesis of AD remain unclear and are hotly debated. One proposal focuses on  $A\beta$  production and deposition, the so-called amyloid cascade hypothesis (Figure 1) [4–6]. This hypothesis posits that increased  $A\beta$  production and deposition plays the key role in triggering neuronal dysfunction and death in AD. Evidence, including  $A\beta$  deposition in AD brain, the toxic properties of  $A\beta$  to neurons *in vitro*, and the identification of mutations in amyloid precursor protein (APP) in familial early onset AD have supported the amyloid cascade hypothesis. Based on this theory, tremendous efforts had been made during the last decade to uncover the mechanisms underlying the production of  $A\beta$ . From these studies it has been shown that sequential cleavage of APP by  $\beta$ -secretase and  $\gamma$ -secretase generates  $A\beta$  peptides (Figure 2) [7, 8]. Indeed, pharmacologic intervention targeted at  $A\beta$  generation through inhibitors of  $\beta$ -site cleaving enzyme (BACE) and  $\gamma$ -secretase is being

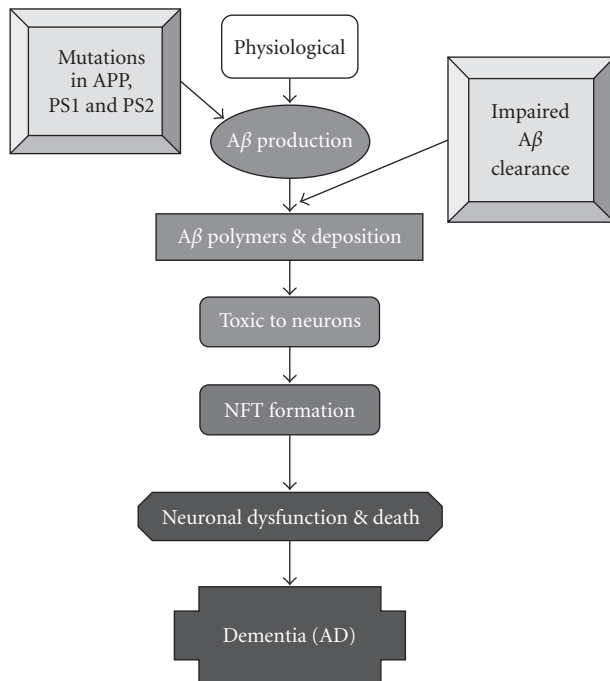


FIGURE 1: Amyloid cascade hypothesis.  $A\beta$  is a normal metabolite which, under physiological conditions, is constantly produced and quickly degraded. Due to genetic defects such as mutations in APP, PS1, or PS2,  $A\beta$  production is increased, resulting in familial AD. A similar phenotype can occur with reduction in the  $A\beta$  catabolic pathways. Accumulating  $A\beta$  will initially oligomerize, gradually form fibrils, and culminate in microscopically visible amyloid plaques. Soluble and fibrillar  $A\beta$  and associated plaque proteins are toxic to neurons, resulting in synaptic loss, the formation of neurofibrillary tangles, and eventual neuronal death and AD [5].

widely pursued [9, 10]. Attempts to block or regulate those two enzymes together with immunotherapy aimed at reducing brain  $A\beta$  have been or will soon be tried in AD patients [9, 11–13].

### CANDIDATE ENZYMES FOR $A\beta$ DEGRADATION

Altering catabolism is another way to reduce  $A\beta$  levels in the brains of AD. Many proteases or peptidases have been reported with the capability of cleaving  $A\beta$  either in vitro or in vivo. These include neprilysin (NEP) [14–16], endothelin-converting enzyme (ECE)-1 [17], insulin-degrading enzyme (IDE) [18–20], angiotensin-converting enzyme (ACE) [21], uPA/tPA-plasmin system [22, 23], cathepsin D [24, 25], gelatinase A [26], gelatinase B [27], matrix metalloendopeptidase-9 [28], coagulation factor XIa [29], antibody light chain c23.5 and hk14 [30], and  $\alpha$ 2-macroglobulin complexes [31]. Many of them have more than one cleavage site in the  $A\beta$  peptide (Figure 3). The basic biological features of these enzymes are summarized in Table 1. There are probably other proteases with potential to cleave  $A\beta$  if all peptide bonds are taken into consideration, but only those physiologically or pathologically relevant are

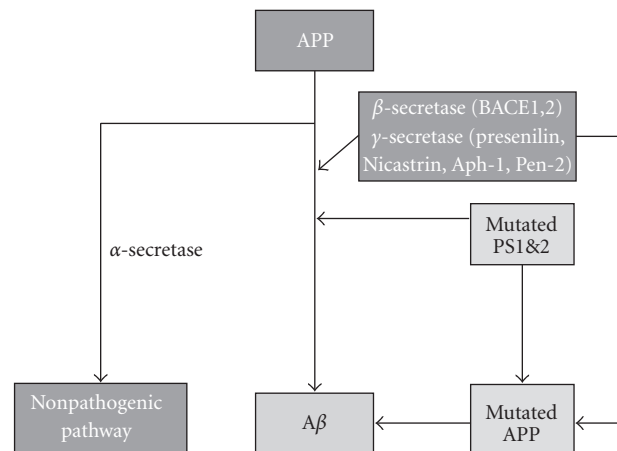


FIGURE 2:  $A\beta$  biogenesis. Normally,  $A\beta$  is derived from the transmembrane region of amyloid precursor protein (APP) through the sequential cleavage by BACE and  $\gamma$ -secretase. Under physiological conditions,  $A\beta$  maintains a steady-state level and is necessary for multiple physiological functions [168]. In AD,  $A\beta$  production is increased due to mutations in APP and/or in presenilin (PS1 and PS2) genes. There is an  $\alpha$ -secretase cleavage site located between  $\beta$ - and  $\gamma$ -secretase cleavage sites that generates soluble, nonpathogenic peptides [8].

discussed. Among them, NEP, IDE, ECE, ACE and plasmin, tissue plasminogen activator (tPA), and urokinase-type plasminogen activator (uPA) system are the most promising  $A\beta$ -degrading candidates.

### NEPRILYSIN (NEP)

NEP is also known as neutral endopeptidase-24.11, EC 3.4.24.11, enkephalinase, neutrophil cluster-differentiation antigen 10 (CD10), or common acute lymphoblastic leukemia antigen (CALLA) [32–38]. In humans, the NEP gene is located on chromosome 3q21–q27 and contains 24 exons [39, 40]. NEP is composed of 750 amino acids with a calculated molecular weight of approximately 86 kDa [41]. Because of abundant posttranslational modifications, especially glycosylation [42], NEP from human brain tissues migrates between 97–110 kDa on denaturing gel electrophoresis. As a plasma membrane-bound glycoprotein, NEP is composed of a short N-terminal cytoplasmic tail, a membrane-spanning domain, and a large C-terminal extracellular catalytic domain. The latter contains a HExxH zinc-binding motif [43, 44], which facilitates the hydrolysis of extracellular oligopeptides (< 5 kDa) on the amino side of hydrophobic residues, such as the small, hydrophobic  $A\beta$ 40 and  $A\beta$ 42 peptides.

NEP is widely expressed in many normal tissues including the brush-border of intestinal and kidney epithelial cells, neutrophils, thymocytes, lung, prostate, testes, and brain [45–49]. In the brain, it is expressed on neuronal plasma membranes, both pre- and postsynaptically [50, 51], and is most abundant in the nigrostriatal pathway, as well as in brain areas vulnerable to amyloid plaque deposition, such as the hippocampus [43, 52].

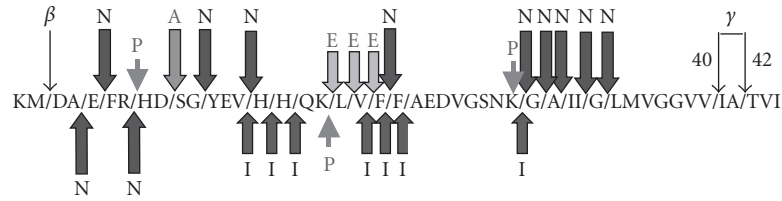


FIGURE 3: Cleavage sites of  $A\beta$  by various enzymes including NEP (N) [14, 169, 170], ECE (E) [17, 169], IDE (I) [170, 171], plasmin (P) [172], and ACE (A) [21].  $\beta$ :  $\gamma$ -secretase;  $\gamma$ :  $\gamma$ -secretase; 40:  $A\beta$ 40; 42:  $A\beta$ 42. Modified from [173].

TABLE 1: Features of selected  $A\beta$ -degrading enzymes.

Protease	a.k.a.	Class	Subcellular location	Substrates in addition to $A\beta$
Neprilysin	CD10, CALLA, EC 3.4.24.15	M	Cellular & intracellular membrane including presynaptic membrane	Enkephalin, cholecystokinin, neuropeptide Y, substance P, opioid peptides, atrial natriuretic peptides, bombesin-like peptides, chemotactic peptides, adrenocorticotropin hormone (ACTH)
Insulin-degrading enzyme	EC 3.4.24.56 Insulysin, IDE	M	Cytosol, cellular, and intracellular membrane extracellular space	Insulin, glucagon, atrial natriuretic factor, $\beta$ -endophin amylin, APP intracellular domain TGF $\alpha$
Endothelin-converting enzyme	EC 3.4.24.71 ECE	M	Trans-Golgi network cell surface	Big endothelin, substance P, bradykinin, oxidized insulin B chain
Angiotensin-converting enzyme	EC 3.4.15.1; ACE; dipeptidyl carboxypeptidase	M	plasma membranes perinuclear region	Angitensin-I, enkaphalins, bradykinin
Plasmin/uPA/tPA	EC 3.4.21.31 tissue plasminogen activator for tPA; urokinase-type plasminogen activator for uPA	S	Extracellular space	fibrin, plasminogon, & other matrix proteins

Note: a.k.a. is also known as M: metalloproteases and S: serine.

The first clue that NEP was involved in  $A\beta$  degradation was provided by Howell et al [14]. Using high-performance liquid chromatography (HPLC) combined with mass spectroscopic analysis, they found that NEP cleaved  $A\beta$  between residues Glu3-Phe4, Gly9-Trp10, Phe19-Phe20, Ala30-Ile31, and Gly33-Leu34. The true breakthrough demonstrating the importance of NEP was demonstration that NEP was the rate-limiting enzyme for  $A\beta$  degradation in vivo made by Iwata et al in 2000 [15]. After injecting radio-labeled  $A\beta$  peptides into rat hippocampus in the presence or absence of various protease inhibitors, the resultant  $A\beta$  fragments were analyzed by HPLC equipped with flow scintillation. Iwata and coworkers found that  $A\beta$ 42 was degraded in the hippocampus, with a half-life of 17.5 minutes and with  $A\beta$ 10–37 as the major catabolic intermediate. Infusion of thiorphan, a specific NEP inhibitor [53], directly into rat hippocampus

for 3 days elevated endogenous  $A\beta$  levels, and infusion for 30 days resulted in further endogenous  $A\beta$  accumulation and accumulation of extracellular  $A\beta$  deposits resembling amyloid plaques [15, 54]. They also found that almost all radio-labeled  $A\beta$ 42 could be recovered from the hippocampus 1 hour after the injection, which suggested that  $A\beta$  clearance depends predominantly on local proteolysis, rather than transport across the blood brain barrier into the blood or into the cerebrospinal fluid [15]. Interestingly, in another independent study, it was found that radio-labeled  $A\beta$ 40 injected into mouse brain was more readily transferred to blood, compared with  $A\beta$ 42, suggesting that the relative contributions of degradation and transport to brain  $A\beta$  clearance might be different for these two peptides [55]. Furthermore, it had been found that NEP was able to degrade not only monomeric, but also oligomeric forms of both

A $\beta$ 40 and A $\beta$ 42 [56], both intracellularly and extracellularly [57].

The role of NEP in A $\beta$  degradation was solidified by studies in transgenic mice. In partially NEP deficient animals, the degradation of both endogenous and exogenous A $\beta$  peptides was tightly correlated with gene dose, suggesting that even partial down-regulation of NEP activity could contribute to A $\beta$  accumulation. These studies also established that NEP is a physiologically relevant A $\beta$  degrading enzyme [16]. On the other hand, overexpression of NEP by gene transfer in amyloid-depositing transgenic mice slowed, and in some cases reversed A $\beta$  deposition [54, 58–60].

Studies in human subjects have also supported the notion that NEP plays a key role in brain A $\beta$  metabolism and AD pathogenesis. As mentioned above, aging is one of the most important risk factors for AD [61] and is associated with the accumulation of A $\beta$  even in cognitively normal elderly [62, 63]. Although systematic study of the relationship between NEP and aging in humans remains to be done, aging mice show region selective decreases in NEP mRNA expression [52, 64, 65]. These changes occurred despite maintenance of synaptic and neuronal numbers suggesting gene specificity. Immunohistochemical studies on AD brains have revealed NEP immunoreactivity in senile plaques [49]. Quantitative analysis showed that both NEP mRNA and protein were significantly lower in AD than in age-matched normal control brains [65–68]. Reductions occurred selectively in the regions most vulnerable to AD pathology, but not in other brain areas such as cerebellum or in peripheral organs [65, 66]. NEP was also decreased in the cerebrospinal fluid (CSF) of prodromal Alzheimer's disease patients [69], consistent with cause and effect. Interestingly, an inverse relationship between NEP and A $\beta$  levels in AD brain vasculature has been reported. These data suggested that NEP may play a role in cerebral amyloid angiopathy (CAA), another very common pathological change found in AD brains [70]. Consistent with these findings, A $\beta$  mutations identified in familial AD found in Dutch, Flemish, Italian, and Arctic families do not increase A $\beta$  production, but rather cause presenile parenchymal amyloidosis and CAA [71].

Recent data from our study showed that NEP decreased in AD brains, but not in pathological aging (PA), a term to describe neurologically normal individuals with high brain amyloid burden (sufficient to diagnose AD with the Khachaturian criteria), but minimal or no neurofibrillary degeneration (Braak neurofibrillary tangle stages of three or less) [63, 72]. Interestingly, NEP levels were inversely correlated with a range of amyloid measures including senile plaque counts and levels of A $\beta$ 40 and A $\beta$ 42 in cortical homogenates. The NEP levels were also correlated with clinical cognitive scores, with highest levels of NEP in those with best performance on clinical measures, regardless of whether or not there were cortical amyloid deposits [72]. These results suggest that the deposition of A $\beta$  in AD and PA brains differs in some way, either quantitatively or qualitatively. The results were not merely due to synaptic loss in AD, but also not in PA as measured by synaptic markers since NEP was not decreased in frontal dementia with decreased synaptic markers.

These data support the hypothesis that decreased NEP contributes to A $\beta$  deposition in AD, but perhaps in means that are not entirely linked to visible amyloid deposition [72], perhaps implicating failed degradation of toxic soluble intermediates in AD.

Taken together, these data indicate that NEP is an important enzyme that contributes to the normal metabolism, accumulation, and perhaps toxicity A $\beta$  in AD.

## ENDOTHELIN-CONVERTING ENZYME (ECE)

Endothelin (ET) is a potent vasoconstrictive peptide produced in vascular endothelial cells [73]. In addition, ET also plays an important role in early development of the neural crest and, thus, organogenesis [74]. Endothelin-converting enzyme (ECE) is a transmembrane metalloprotease that catalyzes the conversion of pro-ET (also referred to big-ET) into vasoactive endothelin. So far, two different isoforms of ECE—ECE-1 and ECE-2—have been cloned in humans [75–77]. It has been estimated that expression of ECE-2 is only 1–2% as much as the more abundant ECE-1 based on comparative mRNA transcript levels in endothelial cells [78]. Studies have suggested that ECE-1, but not ECE-2, is a possible brain A $\beta$ -degrading enzyme [17].

ECE-1 consists of 758 amino acids [79] and is the major enzyme responsible for specific cleavage of biologically inactive pro-ET-1 to active ET-1 in vascular endothelial cells. It is a membrane-bound type II metalloprotease and shares significant sequence identity (about 38% homologue at the amino acid level) with NEP. ECE-1 is abundantly expressed in the vascular endothelial cells of all organs and is also widely expressed in nonvascular cells of many tissues, including lung, pancreas, testis, ovary, adrenal gland, and kidney [75, 80–83]. Recent systematic immunohistochemical analyses have shown ECE-1 widely expressed in human brain, including neurons in the diencephalon, brainstem, basal nuclei, cerebral cortex, cerebellar hemisphere, amygdala, and hippocampus [84, 85]. Four isoforms of ECE-1 have been identified to date [75, 86–90]. All of them are encoded by a single gene located on chromosome 1 (1p36), and they differ in their cytoplasmic tail domains through alternative promoter usage. The four isoforms cleave pro-ET with similar efficiency, but they differ in their tissue distribution and subcellular localization [87, 90]. Human ECE-1a is localized predominantly in plasma membrane. Human ECE-1c and ECE-1d have also been reported to be localized in plasma membrane, but also in intracellular compartments. In contrast, human ECE-1b is expressed exclusively intracellularly, particularly in Golgi-like structures and the cytoplasmic face of the plasma membrane [90–92].

Although both ECE-1 and NEP are metalloendopeptidases and thus subject to competitive inhibition by the metalloprotease inhibitors nanomolar concentrations of thiorphan and phosphoramidon can inhibit NEP, whereas ECE-1 is inhibited only at micromolar concentrations of phosphoramidon, and it is insensitive to thiorphan [53]. Another difference is that ECE-1 is active only at neutral pH, while



NEP is active over a slightly wider pH range (pH 6.5–7.5) [72, 93].

By using HPLC, mass spectrometry, and N-terminal sequence analysis, Eckman and her colleagues provided the first evidence that ECE-1 may be involved in the metabolism of A $\beta$ . They found that ECE-1 expressed in cultured Chinese hamster ovary cells that lack endogenous ECE activity, reduced the concentration of extracellular A $\beta$  by up to 90%. In vitro, recombinant ECE-1 cleaves synthetic A $\beta$ 40 in at least three sites, resulting in formation of A $\beta$  fragments A $\beta$ 1–16, A $\beta$ 1–17, A $\beta$ 1–19, and A $\beta$ 20–40 [17]. In mice deficient for ECE-1 and the closely related ECE-2, both A $\beta$ 40 and A $\beta$ 42 levels were significantly higher when compared with age-matched wild-type littermate controls. Taken together, the results suggest that ECE activity might be an important factor involved in A $\beta$  clearance in vivo [94]. How important is ECE-2 in this process is yet to be determined, and direct evidence that ECE contributes to A $\beta$  deposition in human AD brains remains to be determined.

### INSULIN-DEGRADING ENZYME (IDE)

IDE is also known as EC 3.4.24.56, insulin protease, insulinolysin, or insulinase [95, 96]. Cloned human IDE consists of 1019 amino acids [97]. The IDE gene was mapped to chromosome 10q23–q25, which made it a candidate gene for the Alzheimer disease-6 locus (known as AD6) [98, 99]. It is a zinc metalloendopeptidase that hydrolyzes multiple peptides, including insulin, glucagon, atrial natriuretic factor, transforming growth factor- $\alpha$ ,  $\beta$ -endorphin, amylin, and the APP intracellular domain (AICD) in addition to A $\beta$  [100, 101]. Purified IDE from several mammalian tissues, including blood cells, skeletal muscle, liver, and brain, migrates as a 110 kDa band on denaturing gel electrophoresis, but it migrates as a 300 kDa band under nondenaturing conditions. These results suggest that native IDE exists as a mixture of dimers and tetramers [100, 102]. IDE is active at neutral pH and dimers have greater activity than monomers [96, 103, 104]. Subcellularly, IDE is primarily located in the cytosol, although it also had been found in peroxisomes [105], plasma membrane [106, 107], and in a secreted form [20].

Kurochkin and Goto reported the first evidence that IDE might be involved in A $\beta$  degradation [18]. They found that <sup>125</sup>I-labeled synthetic A $\beta$  specifically cross-linked to a single 110 kDa protein, which was shown to be IDE, in cytosol fractions from rat brain and liver. Purified rat IDE effectively degraded synthetic A $\beta$  in vitro. Subsequently, it was shown that an IDE-like activity from soluble and synaptic membrane fractions from postmortem human and fresh rat brain also degraded A $\beta$  peptides [19, 108]. Studies in the cultured cells also proved that IDE could degrade both endogenous and synthetic A $\beta$  in vitro [20, 109]. The overexpression of IDE in Chinese hamster ovary cells resulted in a marked reduction in levels of intracellular detergent-soluble A $\beta$ , as well as reduced levels of extracellular A $\beta$ 40 and A42 [110].

Transgenic mice overexpressing IDE showed significant reductions of total amyloid burden and improved survival

rates [58], while IDE knockout mice demonstrated a clear elevation of brain A $\beta$  and the APP intracellular domain. Additionally, heterozygous mice exhibited A $\beta$  levels that were intermediate between wild-type controls and knock-out mice, indicating that IDE affected A $\beta$  level in a gene-dose dependent manner [111, 112].

Immunohistochemical studies showed that IDE was primarily expressed in neurons, but was also located in senile plaques, in AD brain [113]. The finding that IDE mRNA and protein were reduced in the hippocampus of AD patients, especially in APOE e4 carriers, suggested that APOE e4 might be sensitive to IDE expression levels with downstream effects on A $\beta$  metabolism [114]. Like NEP, IDE also showed progressively decreased expression that was age- and region-dependent [65]. Thus, strong evidence exists that IDE is another important A $\beta$ -degrading enzyme that may play a role in the amyloid pathology of AD.

### ANGIOTENSIN-CONVERTING ENZYME (ACE)

Angiotensin-converting enzyme, also known as EC 3.4.15.1, dipeptidyl carboxypeptidase, or ACE, is a membrane-bound zinc metalloprotease. At least two ACE isotypes (ACE1 and ACE2) had been cloned in humans, thus far [115]. ACE is composed of 732 amino acids [116] and contains two proteolytically active domains that are located at N- and C-termini, respectively [117]. The major function of ACE is to catalyze the conversion of angiotensin I (AngI) to angiotensin II (AngII), which plays an important role in maintaining blood pressure, body fluid, and sodium homeostasis [118].

ACE is also widely expressed both outside and within the CNS. In the brain, ACE was found at highest levels in circumventricular organs such as the subfornical organ, area postrema, and the median eminence [119]. It was detected in other areas as well, including the caudate nucleus, putamen, substantia nigra pars reticularis, nucleus of the solitary tract (NTS), dorsal motor nucleus, median preoptic nucleus, and choroid plexus in rat, human, rabbit, sheep, monkey [120].

Most of the evidence for the potential relationship between ACE and AD has come from human genetic studies [121–127]. Patients at higher AD risk had an insertion (I) polymorphism within intron 16 of the ACE gene, which was associated with AD [121]. Interestingly, patients with a deletion polymorphism had a lower risk of AD [123, 124]. Genetic analysis of postmortem AD brains showed homozygous I/I was associated with higher brain A $\beta$  levels compared to D/D allele carriers [128]. Results from earlier preclinical and clinical studies suggested that ACE might have a role in the modulation of cognitive memory processes in the rat and in humans [129].

Hu and coworkers provided the first evidence that ACE could significantly inhibit the aggregation, deposition, and cytotoxicity of A $\beta$  in vitro by cleavage of A $\beta$  at Asp7-Ser8. This was a surprising finding given the known specificity of ACE [130] and the failure of ACE inhibitors to alter A $\beta$  degradation in vivo [15, 16]. Whether this discrepancy was due to different experimental systems (eg, in vitro versus in

vivo) is not clear. Further work in other experimental systems such as ACE-deficient or knockout animals is needed to clarify the role ACE might have in amyloid pathology in AD.

A very recent report by Hemming and Selkoe, showed that ACE expression promoted the degradation of endogenous A $\beta$ 40 and A $\beta$ 42 [131]. Using site-directed mutagenesis, they also showed that both N- and C-terminal proteolytically active domains contributed to A $\beta$  degradation. Captopril, a widely prescribed ACE inhibitor blocked A $\beta$  cleavage in culture medium. This is potentially very important observation because it suggests widely used ACE inhibitors could increase cerebral A $\beta$  levels in patients with hypertension.

Unlike other candidate A $\beta$ -degrading enzymes discussed above, the levels of both ACE protein and activity were elevated in postmortem brains [132–134]. Given that other A $\beta$ -degrading enzymes such as NEP and IDE are decreased in AD brains compared to age-matched healthy controls [65–68, 72], ACE may show compensatory up-regulation in response to accumulating A $\beta$ . Along with concurrent evaluation of NEP, ECE, IDE, ACE, and possibly others in the same panel of postmortem human brains with the spectrum of pathology from normal aging, early and advanced AD will be helpful in clarifying respective functions of these proteases.

#### **PLASMIN, TISSUE PLASMINOGEN ACTIVATOR (tPA), AND UROKINASE-TYPE PLASMINOGEN ACTIVATOR (uPA)**

Plasmin is a serine protease important in the degradation of many extracellular matrix components [135]. The principal components of this system include plasminogen/plasmin, tissue plasminogen activator (tPA), urokinase-type plasminogen activator (uPA) [136]. tPA and uPA cleave plasminogen to yield the active serine protease, plasmin. In the nervous system, plasminogen and uPA are expressed in neurons, while tPA is synthesized by neurons and microglial cells [137]. The plasmin system is involved in many normal neural functions, such as neuronal plasticity [138], learning, and memory [139].

Several studies showed that A $\beta$  aggregates could substitute for fibrin aggregates in activating tPA, and suggested that tPA may be activated by A $\beta$  in AD [140, 141]. Later, it was reported that brain plasmin enhanced A $\beta$  degradation [142, 143], while plasmin and its activity were decreased in AD brains [142, 144]. In cultured cells purified plasmin significantly decreased the level of neuronal injury induced by aggregated A $\beta$ , presumably by degrading A $\beta$  [143, 145]. However, the *in vivo* effect of plasmin could be very different given that serum amyloid P, that is associated with amyloid pathology in AD brain, is able to prevent proteolysis of purified cerebral A $\beta$  [146]. Indeed, plasminogen deficient mice did not show increased A $\beta$  in the brain or in the plasma and suggested that plasmin does not regulate steady-state A $\beta$  levels in nonpathologic conditions [147], although it might be involved in the degradation of pathological A $\beta$  aggregates.

#### **OXIDATIVE DAMAGE TO A $\beta$ -DEGRADING ENZYMES**

Some studies have indicated that genetic polymorphisms of A $\beta$ -degrading enzymes including NEP, IDE, ACE might be associated with AD [122, 125, 127, 148–155], although these results remain controversial [128, 156–161]. Further clinical and pathologic studies of large numbers of individuals carrying various mutations in possible A $\beta$ -degrading enzymes are needed to clarify this issue.

In addition to genetics, many environmental factors such as oxidative stress can potentially impair the activity of A $\beta$ -degrading enzymes [162–164]. Recent data showed that NEP and IDE might be substrates for oxidative damage during aging and in AD [65, 68]. Both NEP and IDE from AD brain tissues could be modified by 4-hydroxy-nonenal (HNE), a by-product of lipid peroxidation [165]. The ratio of oxidized NEP from frontal cortex [68] and IDE from hippocampus [65] was greater in AD brains than in age-matched controls. Studies reported by Russo et al failed to confirm these findings [166]. In their study, they found that NEP mRNA from AD brains was significantly lower than in controls, but not NEP protein [166], which was contradictory to several previous reports [65–68]. One possible reason for such a discrepancy might be purely technical, reflecting different immunoprecipitation protocols and incomplete antigenic recovery [166]. Although very recent data confirmed that both recombinant IDE and the extracellular domain of NEP were modified by HNE *in vitro* [167], additional, *in vivo* studies of neuronal proteases are needed to clarify this potentially very important mechanism for A $\beta$  deposition in AD development.

#### **SUMMARY**

Since the majority of AD cases are sporadic without clear genetic causes, and that even a large percentage of familial cases cannot be explained by the overproduction of A $\beta$ , multiple factors are likely involved in the pathogenic metabolism of A $\beta$  in AD (Figure 4). Exploration for possible mechanisms underlying A $\beta$  accumulation in AD is crucial to resolve these issues. There are growing and compelling data now available to implicate A $\beta$  degradation in AD pathogenesis. A $\beta$  is a substrate of a wide range of proteases, which are likely contribute to the accumulation of A $\beta$  in AD. Both enzymatic loss through genetic mutations and nongenetic factors, such as direct oxidative damage or enhanced production of inhibitors, may contribute to aberrant A $\beta$  catabolism. Current results from *in vitro* and animal models support NEP, IDE, ECE, and ACE as probable enzymes for A $\beta$  degradation, but data from humans remain largely missing. Due to clear limitations of animal models, validation in human subjects with AD will be critical to establish the physiologic significance of these proteases. Measurement of brain A $\beta$  levels, amyloid pathology and clinical cognitive performance with enzyme activity, location and expression will help to clarify which of these many enzymes that are capable of cleaving A $\beta$  are actually key players in human disease.

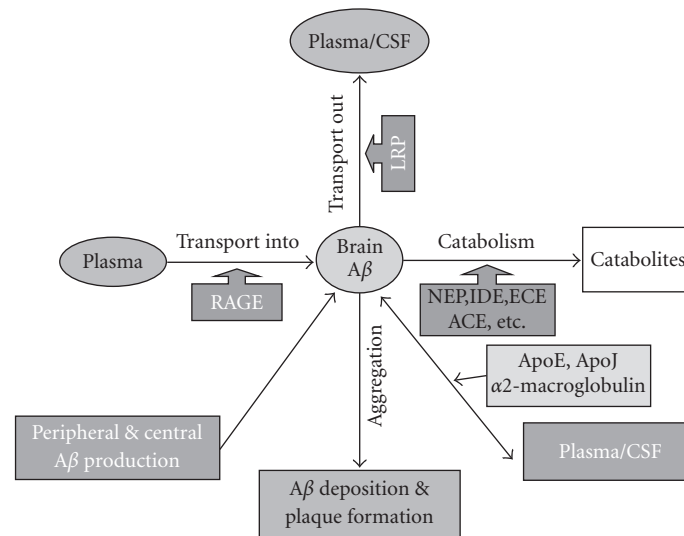


FIGURE 4: Possible brain  $A\beta$  clearance mechanisms.  $A\beta$  peptides may be removed by enzymatic degradation within brain parenchyma [38, 173] or they can be transported through the blood-brain-barrier into the blood or CSF by receptor for advanced glycation endproducts (RAGE), ApoE,  $\beta$ -2-macroglobulin, and the low-density lipoprotein receptor (LRP) [3, 174–176]. The steady-state level of brain  $A\beta$  depends upon a balance between production and catabolism. Increased production (like in familial AD) and/or decreased clearance (for most sporadic AD) will result in elevated brain  $A\beta$  levels and potentially trigger or accelerate the pathogenesis of AD. RAGE: receptor for advanced glycation end products; LRP: low-density lipoprotein receptor-related protein.

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