Review Article

β -Amyloid Degradation and Alzheimer's Disease

Deng-Shun Wang,¹ Dennis W. Dickson,² and James S. Malter^{1,3}

¹ Department of Pathology and Laboratory Medicine, School of Medicine, University of Wisconsin, Madison, WI 53705, USA

² Departments of Pathology (Neuropathology) and Neuroscience, Mayo Clinic College of Medicine, Jacksonville, FL 32224, USA

³ Waisman Center for Developmental Disabilities, School of Medicine, University of Wisconsin, Madison, WI 53705, USA

Received 18 November 2005; Revised 18 January 2006; Accepted 1 February 2006

Extensive β -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.

Copyright © 2006 Deng-Shun Wang et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 β -secretase and γ -secretase generates $A\beta$ peptides (Figure 2) [7, 8]. Indeed, pharmacologic intervention targeted at $A\beta$ generation through inhibitors of β -site cleaving enzyme (BACE) and γ -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 α 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 γ -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 α -secretase cleavage site located between β - and γ -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 Aβ by various enzymes including NEP (N) [14, 169, 170], ECE (E) [17, 169], IDE (I) [170, 171], plasmin (P) [172], and ACE (A) [21]. β: γ-secretase; γ: γ-secretase; 40: Aβ40; 42: Aβ42. Modified from [173].

TABLE 1: Features of selected Aβ-degrading enzymes.

Protease	a.k.a.	Class	Subcellular location	Substrates in addition to Aβ
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, β-endorphin amylin, APP intracellular domain TGFα
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, plasminogen, & other matrix proteins

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

The first clue that NEP was involved in Aβ degradation was provided by Howell et al [14]. Using high-performance liquid chromatography (HPLC) combined with mass spectroscopic analysis, they found that NEP cleaved Aβ 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 Aβ degradation in vivo made by Iwata et al in 2000 [15]. After injecting radio-labeled Aβ peptides into rat hippocampus in the presence or absence of various protease inhibitors, the resultant Aβ fragments were analyzed by HPLC equipped with flow scintillation. Iwata and coworkers found that Aβ42 was degraded in the hippocampus, with a half-life of 17.5 minutes and with Aβ10–37 as the major catabolic intermediate. Infusion of thiorphan, a specific NEP inhibitor [53], directly into rat hippocampus

for 3 days elevated endogenous Aβ levels, and infusion for 30 days resulted in further endogenous Aβ accumulation and accumulation of extracellular Aβ deposits resembling amyloid plaques [15, 54]. They also found that almost all radio-labeled Aβ42 could be recovered from the hippocampus 1 hour after the injection, which suggested that Aβ 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 Aβ40 injected into mouse brain was more readily transferred to blood, compared with Aβ42, suggesting that the relative contributions of degradation and transport to brain Aβ 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 β 40 and A β 42 [56], both intracellularly and extracellularly [57].

The role of NEP in A β degradation was solidified by studies in transgenic mice. In partially NEP deficient animals, the degradation of both endogenous and exogenous A β peptides was tightly correlated with gene dose, suggesting that even partial down-regulation of NEP activity could contribute to A β accumulation. These studies also established that NEP is a physiologically relevant A β 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 β deposition [54, 58–60].

Studies in human subjects have also supported the notion that NEP plays a key role in brain A β 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 β 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 β 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 β mutations identified in familial AD found in Dutch, Flemish, Italian, and Arctic families do not increase A β 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 β 40 and A β 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 β 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 β 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 β 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 β -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 β . They found that ECE-1 expressed in cultured Chinese hamster ovary cells that lack endogenous ECE activity, reduced the concentration of extracellular A β by up to 90%. In vitro, recombinant ECE-1 cleaves synthetic A β 40 in at least three sites, resulting in formation of A β fragments A β 1–16, A β 1–17, A β 1–19, and A β 20–40 [17]. In mice deficient for ECE-1 and the closely related ECE-2, both A β 40 and A β 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 β 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 β 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, insulysin, 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- α , β -endorphin, amylin, and the APP intracellular domain (AICD) in addition to A β [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 involved in A β degradation [18]. They found that ¹²⁵I-labeled synthetic A β 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 β 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 β peptides [19, 108]. Studies in the cultured cells also proved that IDE could degrade both endogenous and synthetic A β 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 β , as well as reduced levels of extracellular A β 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 β and the APP intracellular domain. Additionally, heterozygous mice exhibited A β levels that were intermediate between wild-type controls and knock-out mice, indicating that IDE affected A β 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 β 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 β -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 β 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 β in vitro by cleavage of A β at Asp7-Ser8. This was a surprising finding given the known specificity of ACE [130] and the failure of ACE inhibitors to alter A β 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 β 40 and A β 42 [131]. Using site-directed mutagenesis, they also showed that both N- and C-terminal proteolytically active domains contributed to A β degradation. Captopril, a widely prescribed ACE inhibitor blocked A β cleavage in culture medium. This is potentially very important observation because it suggests widely used ACE inhibitors could increase cerebral A β levels in patients with hypertension.

Unlike other candidate A β -degrading enzymes discussed above, the levels of both ACE protein and activity were elevated in postmortem brains [132–134]. Given that other A β -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 β . 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 β aggregates could substitute for fibrin aggregates in activating tPA, and suggested that tPA may be activated by A β in AD [140, 141]. Later, it was reported that brain plasmin enhanced A β 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 β , presumably by degrading A β [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 β [146]. Indeed, plasminogen deficient mice did not show increased A β in the brain or in the plasma and suggested that plasmin does not regulate steady-state A β levels in nonpathologic conditions [147], although it might be involved in the degradation of pathological A β aggregates.

OXIDATIVE DAMAGE TO A β -DEGRADING ENZYMES

Some studies have indicated that genetic polymorphisms of A β -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 β -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 β -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 β 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 β , multiple factors are likely involved in the pathogenic metabolism of A β in AD (Figure 4). Exploration for possible mechanisms underlying A β accumulation in AD is crucial to resolve these issues. There are growing and compelling data now available to implicate A β degradation in AD pathogenesis. A β is a substrate of a wide range of proteases, which are likely contribute to the accumulation of A β 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 β catabolism. Current results from *in vitro* and animal models support NEP, IDE, ECE, and ACE as probable enzymes for A β 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 β 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 β are actually key players in human disease.

FIGURE 4: Possible brain A β clearance mechanisms. A β 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, β -2-macroglobulin, and the low-density lipoprotein receptor (LRP) [3, 174–176]. The steady-state level of brain A β 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 β levels and potentially trigger or accelerate the pathogenesis of AD. RAGE: receptor for advanced glycation end products; LRP: low-density lipoprotein receptor-related protein.

ACKNOWLEDGMENTS

The authors express their thanks for the financial support from the Research Fund of the Department of Pathology and Laboratory Medicine, University of Wisconsin School of Medicine, Madison to Deng-Shun Wang, NIH-RO1AG10675 and P30HD63352 to James S. Malter, and NIH-P01-AG03949, 5P01AG003949, 5P01AG014449, and 5P50NS040256 to Dennis W. Dickson.

REFERENCES

- [1] Zlokovic BV. Clearing amyloid through the blood-brain barrier. *Journal of Neurochemistry*. 2004;89(4):807–811.
- [2] Zlokovic BV, Deane R, Sallstrom J, Chow N, Miano JM. Neurovascular pathways and Alzheimer amyloid beta-peptide. *Brain Pathology*. 2005;15(1):78–83.
- [3] Zlokovic BV. Neurovascular mechanisms of Alzheimer's neurodegeneration. *Trends in Neurosciences*. 2005;28(4):202–208.
- [4] Glenner GG, Wong CW. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochemical and Biophysical Research Communications*. 1984;120(3):885–890.
- [5] Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*. 2002;297(5580):353–356.
- [6] Scheuner D, Eckman C, Jensen M, et al. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nature Medicine*. 1996;2(8):864–870.
- [7] Mattson MP. Pathways towards and away from Alzheimer's disease. *Nature*. 2004;430(7000):631–639.
- [8] Evin G, Weidemann A. Biogenesis and metabolism of Alzheimer's disease Abeta amyloid peptides. *Peptides*. 2002; 23(7):1285–1297.
- [9] Golde TE. The Abeta hypothesis: leading us to rationally-designed therapeutic strategies for the treatment or prevention of Alzheimer disease. *Brain Pathology*. 2005;15:84–87.
- [10] Eckman EA, Eckman CB. Abeta-degrading enzymes: modulators of Alzheimer's disease pathogenesis and targets for therapeutic intervention. *Biochemical Society Transactions*. 2005;33(pt 5):1101–1105.
- [11] Schenk D, Games D, Seubert P. Potential treatment opportunities for Alzheimer's disease through inhibition of secretases and Abeta immunization. *Journal of Molecular Neuroscience*. 2001;17:259–267.
- [12] Schenk D, Hagen M, Seubert P. Current progress in beta-amyloid immunotherapy. *Current Opinion in immunology*. 2004;16:599–606.
- [13] Bennett DA, Holtzman DM. Immunization therapy for Alzheimer disease? *Neurology*. 2005;64:10–12.
- [14] Howell S, Nalbantoglu J, Crine P. Neutral endopeptidase can hydrolyze beta-amyloid(1-40) but shows no effect on beta-amyloid precursor protein metabolism. *Peptides*. 1995;16: 647–652.
- [15] Iwata N, Tsubuki S, Takaki Y, et al. Identification of the major Abeta1-42-degrading catabolic pathway in brain parenchyma: suppression leads to biochemical and pathological deposition. *Nature Medicine*. 2000;6:143–150.
- [16] Iwata N, Tsubuki S, Takaki Y, et al. Metabolic regulation of brain Abeta by neprilysin. *Science*. 2001;292:1550–1552.
- [17] Eckman EA, Reed DK, Eckman CB. Degradation of the Alzheimer's amyloid beta peptide by endothelin-converting

- enzyme. *The Journal of Biological Chemistry*. 2001;276:24540–24548.
- [18] Kurochkin IV, Goto S. Alzheimer's beta-amyloid peptide specifically interacts with and is degraded by insulin degrading enzyme. *FEBS Letters*. 1994;345:33–37.
 - [19] McDermott JR, Gibson AM. Degradation of Alzheimer's beta-amyloid protein by human and rat brain peptidases: involvement of insulin-degrading enzyme. *Neurochemical Research*. 1997;22:49–56.
 - [20] Qiu WQ, Walsh DM, Ye Z, et al. Insulin-degrading enzyme regulates extracellular levels of amyloid beta-protein by degradation. *The Journal of Biological Chemistry*. 1998;273:32730–32738.
 - [21] Hu J, Igarashi A, Kamata M, Nakagawa H. Angiotensin-converting enzyme degrades Alzheimer amyloid beta-peptide (A beta); retards A beta aggregation, deposition, fibril formation; and inhibits cytotoxicity. *The Journal of Biological Chemistry*. 2001;276:47863–47868.
 - [22] Sasaki H, Saito Y, Hayashi M, Otsuka K, Niwa M. Nucleotide sequence of the tissue-type plasminogen activator cDNA from human fetal lung cells. *Nucleic Acids Research*. 1988;16(12):5692.
 - [23] Verde P, Boast S, Franze A, Robbiati F, Blasi F. An upstream enhancer and a negative element in the 5' flanking region of the human urokinase plasminogen activator gene. *Nucleic Acids Research*. 1988;16:10699–10716.
 - [24] Yamada T, Kluwe-Beckerman B, Liepnieks JJ, Benson MD. In vitro degradation of serum amyloid A by cathepsin D and other acid proteases: possible protection against amyloid fibril formation. *Scandinavian Journal of Immunology*. 1995;41:570–574.
 - [25] Hamazaki H. Cathepsin D is involved in the clearance of Alzheimer's beta-amyloid protein. *FEBS Letters*. 1996;396:139–142.
 - [26] Yamada T, Miyazaki K, Koshikawa N, Takahashi M, Akatsu H, Yamamoto T. Selective localization of gelatinase A, an enzyme degrading beta-amyloid protein, in white matter microglia and in Schwann cells. *Acta Neuropathologica (Berl)*. 1995;89:199–203.
 - [27] Backstrom JR, Lim GP, Cullen MJ, Tokes ZA. Matrix metalloproteinase-9 (MMP-9) is synthesized in neurons of the human hippocampus and is capable of degrading the amyloid-beta peptide (1-40). *The Journal of Neuroscience*. 1996;16:7910–7919.
 - [28] Carvalho KM, Franca MS, Camarao GC, Ruchon AF. A new brain metalloendopeptidase which degrades the Alzheimer beta-amyloid 1-40 peptide producing soluble fragments without neurotoxic effects. *Brazilian Journal of Medical and Biological Research*. 1997;30:1153–1156.
 - [29] Saporito-Irwin SM, Van Nostrand WE. Coagulation factor XIa cleaves the RHDS sequence and abolishes the cell adhesive properties of the amyloid beta-protein. *The Journal of Biological Chemistry*. 1995;270:26265–26269.
 - [30] Rangan SK, Liu R, Brune D, Planque S, Paul S, Sierks MR. Degradation of beta-amyloid by proteolytic antibody light chains. *The Journal of Biochemistry*. 2003;42:14328–14334.
 - [31] Qiu WQ, Borth W, Ye Z, Haass C, Teplow DB, Selkoe DJ. Degradation of amyloid beta-protein by a serine protease-alpha2-macroglobulin complex. *The Journal of Biological Chemistry*. 1996;271:8443–8451.
 - [32] Turner AJ, Tanzawa K. Mammalian membrane metallopeptidases: NEP, ECE, KELL, and PEX. *The FASEB Journal*. 1997;11:355–364.
 - [33] Matsas R, Kenny AJ, Turner AJ. An immunohistochemical study of endopeptidase-24.11 ("enkephalinase") in the pig nervous system. *Neuroscience*. 1986;18:991–1012.
 - [34] Turner AJ. Neuropeptide signalling and cell-surface peptidases. *Advances in Second Messenger and Phosphoprotein Research*. 1990;24:467–471.
 - [35] Sales N, Dutriez I, Maziere B, Ottaviani M, Roques BP. Neutral endopeptidase 24.11 in rat peripheral tissues: comparative localization by 'ex vivo' and 'in vitro' autoradiography. *Regulatory Peptides*. 1991;33:209–222.
 - [36] Letarte M, Vera S, Tran R, et al. Common acute lymphocytic leukemia antigen is identical to neutral endopeptidase. *The Journal of Experimental Medicine*. 1988;168:1247–1253.
 - [37] LeBien TW, McCormack RT. The common acute lymphoblastic leukemia antigen (CD10)—emancipation from a functional enigma. *Blood*. 1989;73:625–635.
 - [38] Iwata N, Higuchi M, Saido TC. Metabolism of amyloid-beta peptide and Alzheimer's disease. *Pharmacology & Therapeutics*. 2005;108:129–148.
 - [39] Roques BP, Noble F, Dauge V, Fournie-Zaluski MC, Beaumont A. Neutral endopeptidase 24.11: structure, inhibition, and experimental and clinical pharmacology. *Pharmacological Reviews*. 1993;45(1):87–146.
 - [40] Barker PE, Shipp MA, D'Adamio L, Masteller EL, Reinherz EL. The common acute lymphoblastic leukemia antigen gene maps to chromosomal region 3 (q21-q27). *Journal of Immunology*. 1989;142:283–287.
 - [41] Malfroy B, Kuang WJ, Seeburg PH, Mason AJ, Schofield PR. Molecular cloning and amino acid sequence of human enkephalinase (neutral endopeptidase). *FEBS Letters*. 1988;229(1):206–210.
 - [42] Lafrance MH, Vezina C, Wang Q, Boileau G, Crine P, Lemay G. Role of glycosylation in transport and enzymic activity of neutral endopeptidase-24.11. *The Biochemical Journal*. 1994;302(pt 2):451–454.
 - [43] Barnes K, Doherty S, Turner AJ. Endopeptidase-24.11 is the integral membrane peptidase initiating degradation of somatostatin in the hippocampus. *Journal of Neurochemistry*. 1995;64:1826–1832.
 - [44] Turner AJ, Isaac RE, Coates D. The neprilysin (NEP) family of zinc metalloendopeptidases: genomics and function. *BioEssays*. 2001;23:261–269.
 - [45] Erdos EG, Skidgel RA. Human neutral endopeptidase 24.11 (NEP, enkephalinase); function, distribution and release. *Advances in Experimental Medicine and Biology*. 1988;240:13–21.
 - [46] Erdos EG, Skidgel RA. Neutral endopeptidase 24.11 (enkephalinase) and related regulators of peptide hormones. *The FASEB Journal*. 1989;3:145–151.
 - [47] Connelly JC, Skidgel RA, Schulz WW, Johnson AR, Erdos EG. Neutral endopeptidase 24.11 in human neutrophils: cleavage of chemotactic peptide. *Proceedings of the National Academy of Sciences of the United States of America*. 1985;82(24):8737–8741.
 - [48] Pierart ME, Najdovski T, Appelboom TE, Deschodt-Lanckman MM. Effect of human endopeptidase 24.11 ("enkephalinase") on IL-1-induced thymocyte proliferation activity. *Journal of Immunology*. 1988;140:3808–3811.
 - [49] Akiyama H, Kondo H, Ikeda K, Kato M, McGeer PL. Immunohistochemical localization of neprilysin in the human cerebral cortex: inverse association with vulnerability to amyloid beta-protein (Abeta) deposition. *Brain Research*. 2001;902:277–281.

- [50] Barnes K, Turner AJ, Kenny AJ. Membrane localization of endopeptidase-24.11 and peptidyl dipeptidase a (angiotensin converting enzyme) in the pig brain: a study using subcellular fractionation and electron microscopic immunocytochemistry. *Journal of Neurochemistry*. 1992;58:2088–2096.
- [51] Fukami S, Watanabe K, Iwata N, et al. Abeta-degrading endopeptidase, neprilysin, in mouse brain: synaptic and axonal localization inversely correlating with Abeta pathology. *Neuroscience Research*. 2002;43:39–56.
- [52] Iwata N, Takaki Y, Fukami S, Tsubuki S, Saido TC. Region-specific reduction of a beta-degrading endopeptidase, neprilysin, in mouse hippocampus upon aging. *Journal of Neuroscience Research*. 2002;70:493–500.
- [53] Takaki Y, Iwata N, Tsubuki S, et al. Biochemical identification of the neutral endopeptidase family member responsible for the catabolism of amyloid beta peptide in the brain. *Journal of Biochemistry (Tokyo)*. 2000;128:897–902.
- [54] Marr RA, Guan H, Rockenstein E, et al. Neprilysin regulates amyloid Beta peptide levels. *Journal of Molecular Neuroscience*. 2004;22:5–11.
- [55] Ji Y, Permanne B, Sigurdsson EM, Holtzman DM, Wisniewski T. Amyloid beta40/42 clearance across the blood-brain barrier following intra-ventricular injections in wild-type, apoE knock-out and human apoE3 or E4 expressing transgenic mice. *Journal of Alzheimer's Disease*. 2001;3:23–30.
- [56] Kanemitsu H, Tomiyama T, Mori H. Human neprilysin is capable of degrading amyloid beta peptide not only in the monomeric form but also the pathological oligomeric form. *Neuroscience Letters*. 2003;350:113–116.
- [57] Hama E, Shirohara K, Iwata N, Saido TC. Effects of neprilysin chimeric proteins targeted to subcellular compartments on amyloid beta peptide clearance in primary neurons. *The Journal of Biological Chemistry*. 2004;279:30259–30264.
- [58] Leissring MA, Farris W, Chang AY, et al. Enhanced proteolysis of beta-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. *Neuron*. 2003;40:1087–1093.
- [59] Mohajeri MH, Kuehnle K, Li H, Poirier R, Tracy J, Nitsch RM. Anti-amyloid activity of neprilysin in plaque-bearing mouse models of Alzheimer's disease. *FEBS Letters*. 2004;562:16–21.
- [60] Marr RA, Rockenstein E, Mukherjee A, et al. Neprilysin gene transfer reduces human amyloid pathology in transgenic mice. *The Journal of Neuroscience*. 2003;23:1992–1996.
- [61] Ott A, Breteler MM, van Harskamp F, et al. Prevalence of Alzheimer's disease and vascular dementia: association with education. The Rotterdam study. *British Medical Journal*. 1995;310:970–973.
- [62] Funato H, Yoshimura M, Kusui K, et al. Quantitation of amyloid beta-protein (A beta) in the cortex during aging and in Alzheimer's disease. *The American Journal of Pathology*. 1998;152:1633–1640.
- [63] Dickson DW, Crystal HA, Mattiace LA, et al. Identification of normal and pathological aging in prospectively studied nondemented elderly humans. *Neurobiology of Aging*. 1992;13:179–189.
- [64] Apelt J, Ach K, Schliebs R. Aging-related down-regulation of neprilysin, a putative beta-amyloid-degrading enzyme, in transgenic Tg2576 Alzheimer-like mouse brain is accompanied by an astroglial upregulation in the vicinity of beta-amyloid plaques. *Neuroscience Letters*. 2003;339:183–186.
- [65] Caccamo A, Oddo S, Sugarman MC, Akbari Y, LaFerla FM. Age- and region dependent alterations in Abeta-degrading enzymes: implications for Abeta-induced disorders. *Neurobiology of Aging*. 2005;26:645–654.
- [66] Yasojima K, Akiyama H, McGeer EG, McGeer PL. Reduced neprilysin in high plaque areas of Alzheimer brain: a possible relationship to deficient degradation of beta-amyloid peptide. *Neuroscience Letters*. 2001;297:97–100.
- [67] Yasojima K, McGeer EG, McGeer PL. Relationship between beta amyloid peptide generating molecules and neprilysin in Alzheimer disease and normal brain. *Brain Research*. 2001;919:115–121.
- [68] Wang D-S, Iwata N, Hama E, Saido TC, Dickson DW. Oxidized neprilysin in aging and Alzheimer's disease brains. *Biochemical and Biophysical Research Communications*. 2003;310:236–241.
- [69] Maruyama M, Higuchi M, Takaki Y, et al. Cerebrospinal fluid neprilysin is reduced in prodromal Alzheimer's disease. *Annals of Neurology*. 2005;57(6):832–842.
- [70] Carpentier M, Robitaille Y, DesGroseillers L, Boileau G, Marcinkiewicz M. Declining expression of neprilysin in Alzheimer disease vasculature: possible involvement in cerebral amyloid angiopathy. *Journal of Neuropathology and Experimental Neurology*. 2002;61:849–856.
- [71] Tsubuki S, Takaki Y, Saido TC. Dutch, Flemish, Italian, and Arctic mutations of APP and resistance of Abeta to physiologically relevant proteolytic degradation. *Lancet*. 2003;361(9373):1957–1958.
- [72] Wang D-S, Lipton RB, Katz MJ, et al. Decreased neprilysin immunoreactivity in Alzheimer disease, but not in pathological aging. *Journal of Neuropathology and Experimental Neurology*. 2005;64:378–385.
- [73] Macours N, Poels J, Hens K, Francis C, Huybrechts R. Structure, evolutionary conservation, and functions of angiotensin- and endothelin-converting enzymes. *International Review of Cytology*. 2004;239:47–97.
- [74] Masaki T. Historical review: endothelin. *Trends in Pharmacological Sciences*. 2004;25:219–224.
- [75] Xu D, Emoto N, Giaid A, et al. ECE-1: a membrane-bound metalloprotease that catalyzes the proteolytic activation of big endothelin-1. *Cell*. 1994;78:473–485.
- [76] Turner AJ, Murphy LJ. Molecular pharmacology of endothelin converting enzymes. *Biochemical Pharmacology*. 1996;51:91–102.
- [77] Lorenzo MN, Khan RY, Wang Y, et al. Human endothelin converting enzyme-2 (ECE2): characterization of mRNA species and chromosomal localization. *Biochimica et Biophysica Acta*. 2001;1522:46–52.
- [78] Emoto N, Yanagisawa M. Endothelin-converting enzyme-2 is a membrane-bound, phosphoramidon-sensitive metalloprotease with acidic pH optimum. *The Journal of Biological Chemistry*. 1995;270:15262–15268.
- [79] Shimada K, Matsushita Y, Wakabayashi K, et al. Cloning and functional expression of human endothelin-converting enzyme cDNA. *Biochemical and Biophysical Research Communications*. 1995;207:807–812.
- [80] Davenport AP, Kuc RE. Cellular expression of isoforms of endothelin-converting enzyme-1 (ECE-1c, ECE-1b and ECE-1a) and endothelin-converting enzyme-2. *Journal of Cardiovascular Pharmacology*. 2000;36:S12–S14.
- [81] Korth P, Bohle RM, Corvol P, Pinet F. Cellular distribution of endothelin converting enzyme-1 in human tissues. *The Journal of Histochemistry and Cytochemistry*. 1999;47:447–462.
- [82] Pupilli C, Romagnani P, Lasagni L, et al. Localization of endothelin-converting enzyme-1 in human kidney. *The American Journal of Physiology*. 1997;273:F749–F756.

- [83] Barnes K, Murphy LJ, Takahashi M, Tanzawa K, Turner AJ. Localization and biochemical characterization of endothelin-converting enzyme. *Journal of Cardiovascular Pharmacology*. 1995;26(suppl 3):S37–S39.
- [84] Naidoo V, Naidoo S, Raidoo DM. Immunolocalisation of endothelin-1 in human brain. *Journal of Chemical Neuroanatomy*. 2004;27:193–200.
- [85] Funalot B, Ouimet T, Claparon A, et al. Endothelinconverting enzyme-1 is expressed in human cerebral cortex and protects against Alzheimer's disease. *Molecular Psychiatry*. 2004; 9:1122–1128, 1059.
- [86] Valdenaire O, Rohrbacher E, Mattei MG. Organization of the gene encoding the human endothelin-converting enzyme (ECE-1). *The Journal of Biological Chemistry*. 1995;270: 29794–29798.
- [87] Valdenaire O, Lepaillier-Enouf D, Egidy G, et al. A fourth isoform of endothelin-converting enzyme (ECE-1) is generated from an additional promoter molecular cloning and characterization. *European Journal of Biochemistry*. 1999;264: 341–349.
- [88] Shimada K, Takahashi M, Ikeda M, Tanzawa K. Identification and characterization of two isoforms of an endothelin-converting enzyme-1. *FEBS Letters*. 1995;371(2):140–144.
- [89] Schmidt M, Kroger B, Jacob E, et al. Molecular characterization of human and bovine endothelin converting enzyme (ECE-1). *FEBS Letters*. 1994;356(2-3):238–243.
- [90] Schweizer A, Valdenaire O, Nelbock P, et al. Human endothelin-converting enzyme (ECE-1): three isoforms with distinct subcellular localizations. *The Biochemical Journal*. 1997;328(pt 3):871–877.
- [91] Cailler F, Zappulla JP, Boileau G, Crine P. The N-terminal segment of endothelin-converting enzyme (ECE)-1b contains a di-leucine motif that can redirect neprilysin to an intracellular compartment in Madin-Darby canine kidney (MDCK) cells. *The Biochemical Journal*. 1999;341(pt 1):119–126.
- [92] Azarani A, Boileau G, Crine P. Recombinant human endothelin-converting enzyme ECE-1b is located in an intracellular compartment when expressed in polarized Madin-Darby canine kidney cells. *The Biochemical Journal*. 1998;333(pt 2):439–448.
- [93] Fahnoe DC, Knapp J, Johnson GD, Ahn K. Inhibitor potencies and substrate preference for endothelin-converting enzyme-1 are dramatically affected by pH. *Journal of Cardiovascular Pharmacology*. 2000;36(5 suppl 1):S22–S25.
- [94] Eckman EA, Watson M, Marlow L, Sambamurti K, Eckman CB. Alzheimer's disease beta-amyloid peptide is increased in mice deficient in endothelin-converting enzyme. *The Journal of Biological Chemistry*. 2003;278(4):2081–2084.
- [95] Duckworth WC, Stentz FB, Heinemann M, Kitabchi AE. Initial site of insulin cleavage by insulin protease. *Proceedings of the National Academy of Sciences of the United States of America*. 1979;76(2):635–639.
- [96] Song ES, Juliano MA, Juliano L, Hersh LB. Substrate activation of insulin-degrading enzyme (insulysin). A potential target for drug development. *The Journal of Biological Chemistry*. 2003;278(50):49789–49794.
- [97] Affholter JA, Fried VA, Roth RA. Human insulin-degrading enzyme shares structural and functional homologies with E. coli protease III. *Science*. 1988;242(4884):1415–1418.
- [98] Affholter JA, Hsieh CL, Francke U, Roth RA. Insulin-degrading enzyme: stable expression of the human complementary DNA, characterization of its protein product, and chromosomal mapping of the human and mouse genes. *Molecular Endocrinology*. 1990;4(8):1125–1135.
- [99] Espinosa R 3rd, Lemons RS, Perlman RK, Kuo WL, Rosner MR, Le Beau MM. Localization of the gene encoding insulin-degrading enzyme to human chromosome 10, bands q23–q25. *Cytogenetics and Cell Genetics*. 1991;57(4):184–186.
- [100] Duckworth WC, Bennett RG, Hamel FG. Insulin degradation: progress and potential. *Endocrine Reviews*. 1998;19(5): 608–624.
- [101] Selkoe DJ. Clearing the brain's amyloid cobwebs. *Neuron*. 2001;32(2):177–180.
- [102] Authier F, Posner BI, Bergeron JJ. Insulin-degrading enzyme. *Clinical and Investigative Medicine*. 1996;19:149–160.
- [103] Mirsky IA, Kaplan S, Broh-Kahn RH. Persinogen excretion (uropepsin as an index of the influence of various life situations on gastric secretion. *Research Publications - Association for Research in Nervous and Mental Disease*. 1949;29:628–646.
- [104] Kurochkin IV. Insulin-degrading enzyme: embarking on amyloid destruction. *Trends in Biochemical Sciences*. 2001; 26(7):421–425.
- [105] Duckworth WC, Bennett RG, Hamel FG. Insulin acts intracellularly on proteasomes through insulin-degrading enzyme. *Biochemical and Biophysical Research Communications*. 1998;244(2):390–394.
- [106] Seta KA, Roth RA. Overexpression of insulin degrading enzyme: cellular localization and effects on insulin signaling. *Biochemical and Biophysical Research Communications*. 1997; 231(1):167–171.
- [107] Vekrellis K, Ye Z, Qiu WQ, et al. Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. *The Journal of Neuroscience*. 2000;20(5): 1657–1665.
- [108] Perez A, Morelli L, Cresto JC, Castano EM. Degradation of soluble amyloid beta-peptides 1-40, 1-42, and the Dutch variant 1-40Q by insulin degrading enzyme from Alzheimer disease and control brains. *Neurochemical Research*. 2000; 25(2):247–255.
- [109] Qiu WQ, Ye Z, Kholodenko D, Seubert P, Selkoe DJ. Degradation of amyloid beta-protein by a metalloprotease secreted by microglia and other neural and non-neural cells. *The Journal of Biological Chemistry*. 1997;272(10):6641–6646.
- [110] Sudoh S, Frosch MP, Wolf BA. Differential effects of proteases involved in intracellular degradation of amyloid beta-protein between detergent-soluble and -insoluble pools in CHO-695 cells. *Biochemistry*. 2002;41(4):1091–1099.
- [111] Farris W, Mansourian S, Chang Y, et al. Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. *Proceedings of the National Academy of Sciences of the United States of America*. 2003;100(7):4162–4167.
- [112] Farris W, Mansourian S, Leissring MA, et al. Partial loss-of-function mutations in insulin-degrading enzyme that induce diabetes also impair degradation of amyloid beta-protein. *The American Journal of Pathology*. 2004;164(4):1425–1434.
- [113] Bernstein HG, Ansorge S, Riederer P, Reiser M, Frolich L, Bogerts B. Insulin-degrading enzyme in the Alzheimer's disease brain: prominent localization in neurons and senile plaques. *Neuroscience Letters*. 1999;263(2-3):161–164.
- [114] Cook DG, Leverenz JB, McMillan PJ, et al. Reduced hippocampal insulin-degrading enzyme in late-onset Alzheimer's disease is associated with the apolipoprotein E-epsilon4 allele. *The American Journal of Pathology*. 2003; 162(1):313–319.

- [115] Guy JL, Lambert DW, Warner FJ, Hooper NM, Turner AJ. Membrane-associated zinc peptidase families: comparing ACE and ACE. *Biochimica et Biophysica Acta*. 2005;1751(1):2–8.
- [116] Ehlers MR, Fox EA, Strydom DJ, Riordan JF. Molecular cloning of human testicular angiotensin-converting enzyme: the testis isozyme is identical to the C-terminal half of endothelial angiotensin-converting enzyme. *Proceedings of the National Academy of Sciences of the United States of America*. 1989;86(20):7741–7745.
- [117] Wei L, Alhenc-Gelas F, Corvol P, Clauser E. The two homologous domains of human angiotensin I-converting enzyme are both catalytically active. *The Journal of Biological Chemistry*. 1991;266(14):9002–9008.
- [118] Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *Journal of Clinical Investigation*. 1990;86(4):1343–1346.
- [119] Saavedra JM, Chevillard C. Angiotensin-converting enzyme is present in the subfornical organ and other circumventricular organs of the rat. *Neuroscience Letters*. 1982;29(2):123–127.
- [120] McKinley MJ, Albiston AL, Allen AM, et al. The brain renin-angiotensin system: location and physiological roles. *The International Journal of Biochemistry & Cell Biology*. 2003;35(6):901–918.
- [121] Kehoe PG, Russ C, McIlory S, et al. Variation in DCP1, encoding ACE, is associated with susceptibility to Alzheimer disease. *Nature Genetics*. 1999;21(1):71–72.
- [122] Farrer LA, Sherbatich T, Keryanov SA, et al. Association between angiotensin-converting enzyme and Alzheimer disease. *Archives of Neurology*. 2000;57(2):210–214.
- [123] Elkins JS, Douglas VC, Johnston SC. Alzheimer disease risk and genetic variation in ACE: a meta-analysis. *Neurology*. 2004;62(3):363–368.
- [124] Lehmann DJ, Cortina-Borja M, Warden DR, et al. Large meta-analysis establishes the ACE insertion-deletion polymorphism as a marker of Alzheimer's disease. *American Journal of Epidemiology*. 2005;162(4):305–317.
- [125] Zhang JW, Li XQ, Zhang ZX, et al. Association between angiotensin-converting enzyme gene polymorphism and Alzheimer's disease in a Chinese population. *Dementia and Geriatric Cognitive Disorders*. 2005;20(1):52–56.
- [126] Kolsch H, Jessen F, Freymann N, et al. ACE I/D polymorphism is a risk factor of Alzheimer's disease but not of vascular dementia. *Neuroscience Letters*. 2005;377(1):37–39.
- [127] Narain Y, Yip A, Murphy T, et al. The ACE gene and Alzheimer's disease susceptibility. *Journal of Medical Genetics*. 2000;37(9):695–697.
- [128] Lendon CL, Thaker U, Harris JM, et al. The angiotensin 1-converting enzyme insertion (I)/deletion (D) polymorphism does not influence the extent of amyloid or tau pathology in patients with sporadic Alzheimer's disease. *Neuroscience Letters*. 2002;328(3):314–318.
- [129] Sudilovsky A, Turnbull B, Croog SH, Crook T. Angiotensin converting enzyme and memory: preclinical and clinical data. *International Journal of Neurology*. 1987;21–22:145–162.
- [130] Turner AJ, Hooper NM. The angiotensin-converting enzyme gene family: genomics and pharmacology. *Trends in Pharmacological Sciences*. 2002;23(4):177–183.
- [131] Hemming ML, Selkoe DJ. Amyloid beta-protein is degraded by cellular angiotensin-converting enzyme (ACE) and elevated by an ACE inhibitor. *The Journal of Biological Chemistry*. 2005;280(45):37644–37650.
- [132] Barnes NM, Cheng CH, Costall B, Naylor RJ, Williams TJ, Wischik CM. Angiotensin converting enzyme density is increased in temporal cortex from patients with Alzheimer's disease. *European Journal of Pharmacology*. 1991;200(2–3):289–292.
- [133] Savaskan E, Hock C, Olivieri G, et al. Cortical alterations of angiotensin converting enzyme, angiotensin II and AT1 receptor in Alzheimer's dementia. *Neurobiology of Aging*. 2001;22(4):541–546.
- [134] Arregui A, Perry EK, Rossor M, Tomlinson BE. Angiotensin converting enzyme in Alzheimer's disease increased activity in caudate nucleus and cortical areas. *Journal of Neurochemistry*. 1982;38(5):1490–1492.
- [135] Werb Z. ECM and cell surface proteolysis: regulating cellular ecology. *Cell*. 1997;91(4):439–442.
- [136] Henkin J, Marcotte P, Yang HC. The plasminogen-plasmin system. *Progress in Cardiovascular Diseases*. 1991;34(2):135–164.
- [137] Strickland S, Gualandris A, Rogove AD, Tsirka SE. Extracellular proteases in neuronal function and degeneration. *Cold Spring Harbor Symposia on Quantitative Biology*. 1996;61:739–745.
- [138] Tsirka SE, Rogove AD, Strickland S. Neuronal cell death and tPA. *Nature*. 1996;384(6605):123–124.
- [139] Madani R, Nef S, Vassalli JD. Emotions are building up in the field of extracellular proteolysis. *Trends in Molecular Medicine*. 2003;9(5):183–185.
- [140] Kingston IB, Castro MJ, Anderson S. In vitro stimulation of tissue-type plasminogen activator by Alzheimer amyloid beta-peptide analogues. *Nature Medicine*. 1995;1(2):138–142.
- [141] Wnendt S, Wetzels I, Gunzler WA. Amyloid beta peptides stimulate tissue-type plasminogen activator but not recombinant prourokinase. *Thrombosis Research*. 1997;85(3):217–224.
- [142] Ledesma MD, Da Silva JS, Crassaerts K, Delacourte A, De Strooper B, Dotti CG. Brain plasmin enhances APP alpha-cleavage and Abeta degradation and is reduced in Alzheimer's disease brains. *EMBO Reports*. 2000;1(6):530–535.
- [143] Tucker HM, Kihiko-Ehmann M, Wright S, Rydel RE, Estus S. Tissue plasminogen activator requires plasminogen to modulate amyloid-beta neurotoxicity and deposition. *Journal of Neurochemistry*. 2000;75(5):2172–2177.
- [144] Ledesma MD, Abad-Rodriguez J, Galvan C, et al. Raft disorganization leads to reduced plasmin activity in Alzheimer's disease brains. *EMBO Reports*. 2003;4(12):1190–1196.
- [145] Tucker HM, Kihiko-Ehmann M, Estus S. Urokinase-type plasminogen activator inhibits amyloid-beta neurotoxicity and fibrillogenesis via plasminogen. *Journal of Neuroscience Research*. 2002;70(2):249–255.
- [146] Tennent GA, Lovat LB, Pepys MB. Serum amyloid P component prevents proteolysis of the amyloid fibrils of Alzheimer disease and systemic amyloidosis. *Proceedings of the National Academy of Sciences of the United States of America*. 1995;92(10):4299–4303.
- [147] Tucker HM, Simpson J, Kihiko-Ehmann M, et al. Plasmin deficiency does not alter endogenous murine amyloid beta levels in mice. *Neuroscience Letters*. 2004;368(3):285–289.
- [148] Clarimon J, Munoz FJ, Boada M, et al. Possible increased risk for Alzheimer's disease associated with neprilysin gene. *Journal of Neural Transmission*. 2003;110(6):651–657.

- [149] Helisalmi S, Hiltunen M, Vepsäläinen S, et al. Polymorphisms in neprilysin gene affect the risk of Alzheimer's disease in Finnish patients. *Journal of Neurology, Neurosurgery, and Psychiatry*. 2004;75(12):1746–1748.
- [150] Sakai A, Ujike H, Nakata K, et al. Association of the Neprilysin gene with susceptibility to late-onset Alzheimer's disease. *Dementia and Geriatric Cognitive Disorders*. 2004;17(3):164–169.
- [151] Yang JD, Feng G, Zhang J, et al. Association between angiotensin-converting enzyme gene and late onset Alzheimer's disease in Han Chinese. *Neuroscience Letters*. 2000;295(1-2):41–44.
- [152] Bian L, Yang JD, Guo TW, et al. Insulin-degrading enzyme and Alzheimer disease: a genetic association study in the Han Chinese. *Neurology*. 2004;63(2):241–245.
- [153] Shi J, Zhang S, Tang M, et al. Mutation screening and association study of the neprilysin gene in sporadic Alzheimer's disease in Chinese persons. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*. 2005;60(3):301–306.
- [154] Sleegers K, den Heijer T, van Dijk EJ, et al. ACE gene is associated with Alzheimer's disease and atrophy of hippocampus and amygdala. *Neurobiology of Aging*. 2005;26(8):1153–1159.
- [155] Morelli L, Llovera RE, Alonso LG, et al. Insulin-degrading enzyme degrades amyloid peptides associated with British and Danish familial dementia. *Biochemical and Biophysical Research Communications*. 2005;332(3):808–816.
- [156] Lilius L, Forsell C, Axelman K, Winblad B, Graff C, Tjernberg L. No association between polymorphisms in the neprilysin promoter region and Swedish Alzheimer's disease patients. *Neuroscience Letters*. 2003;337(2):111–113.
- [157] Sakai A, Ujike H, Nakata K, et al. No association between the insulin degrading enzyme gene and Alzheimer's disease in a Japanese population. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*. 2004;125(1):87–91.
- [158] Nowotny P, Hinrichs AL, Smemo S, et al. Association studies between risk for late-onset Alzheimer's disease and variants in insulin degrading enzyme. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics*. 2005;136(1):62–68.
- [159] Monastero R, Caldarella R, Mannino M, et al. Lack of association between angiotensin converting enzyme polymorphism and sporadic Alzheimer's disease. *Neuroscience Letters*. 2002;335(2):147–149.
- [160] Boussaha M, Hannequin D, Verpillat P, Brice A, Frebourg T, Campion D. Polymorphisms of insulin degrading enzyme gene are not associated with Alzheimer's disease. *Neuroscience Letters*. 2002;329(1):121–123.
- [161] Sodeyama N, Mizusawa H, Yamada M, Itoh Y, Otomo E, Matsushita M. Lack of association of neprilysin polymorphism with Alzheimer's disease and Alzheimer's disease-type neuropathological changes. *Journal of Neurology, Neurosurgery, and Psychiatry*. 2001;71(6):817–818.
- [162] Smith MA, Perry G, Richey PL, et al. Oxidative damage in Alzheimer's. *Nature*. 1996;382(6587):120–121.
- [163] Zhu X, Raina AK, Lee HG, et al. Oxidative stress and neuronal adaptation in Alzheimer disease: the role of SAPK pathways. *Antioxidants & Redox Signaling*. 2003;5(5):571–576.
- [164] Smith MA, Sayre LM, Monnier VM, Perry G. Oxidative post-translational modifications in Alzheimer disease. A possible pathogenic role in the formation of senile plaques and neurofibrillary tangles. *Molecular and Chemical Neuropathology*. 1996;28(1-3):41–48.
- [165] Uchida K. 4-Hydroxy-2-nonenal: a product and mediator of oxidative stress. *Progress in Lipid Research*. 2003;42(4):318–343.
- [166] Russo R, Borghi R, Markesbery W, Tabaton M, Piccini A. Neprilysin decreases uniformly in Alzheimer's disease and in normal aging. *FEBS Letters*. 2005;579(27):6027–6030.
- [167] Shinall H, Song ES, Hersh LB. Susceptibility of amyloid beta peptide degrading enzymes to oxidative damage: a potential Alzheimer's disease spiral. *Biochemistry*. 2005;44(46):15345–15350.
- [168] Selkoe DJ. Physiological production of the beta-amyloid protein and the mechanism of Alzheimer's disease. *Trends in Neurosciences*. 1993;16(10):403–409.
- [169] Johnson GD, Stevenson T, Ahn K. Hydrolysis of peptide hormones by endothelin-converting enzyme-1. A comparison with neprilysin. *The Journal of Biological Chemistry*. 1999;274(7):4053–4058.
- [170] Leissring MA, Lu A, Condrón MM, et al. Kinetics of amyloid beta-protein degradation determined by novel fluorescence- and fluorescence polarization-based assays. *The Journal of Biological Chemistry*. 2003;278(39):37314–37320.
- [171] Mukherjee A, Song E, Kihiko-Ehmann M, et al. Insulysin hydrolyzes amyloid beta peptides to products that are neither neurotoxic nor deposit on amyloid plaques. *The Journal of Neuroscience*. 2000;20(23):8745–8749.
- [172] Van Nostrand WE, Porter M. Plasmin cleavage of the amyloid beta-protein: alteration of secondary structure and stimulation of tissue plasminogen activator activity. *Biochemistry*. 1999;38(35):11570–11576.
- [173] Carson JA, Turner AJ. Beta-amyloid catabolism: roles for neprilysin (NEP) and other metallopeptidases? *Journal of Neurochemistry*. 2002;81(1):1–8.
- [174] Deane R, Wu Z, Sagare A, et al. LRP/amyloid beta-peptide interaction mediates differential brain efflux of Aβ isoforms. *Neuron*. 2004;43(3):333–344.
- [175] Deane R, Wu Z, Zlokovic BV. RAGE (yin) versus LRP (yang) balance regulates Alzheimer amyloid beta-peptide clearance through transport across the blood-brain barrier. *Stroke*. 2004;35(11 suppl 1):2628–2631.
- [176] Deane R, Du Yan S, Subramanyam RK, et al. RAGE mediates amyloid-beta peptide transport across the blood-brain barrier and accumulation in brain. *Nature Medicine*. 2003;9(7):907–913.