Neurochemical Research, Vol. 27, No. 11, November 2002 (© 2002), pp. 1387–1399

The Degradation of Amyloid β as a Therapeutic Strategy in Alzheimer's Disease and Cerebrovascular Amyloidoses*

Laura Morelli,[1] Ramiro Llovera,[1] Sandra Ibendahl,[1] and Eduardo M. Castaño[1,2]

(Accepted April 18, 2002)

The deposition of 4-kDa amyloid β peptide in the brain is a prominent feature of several human diseases. Such process is heterogeneous in terms of causative factors, biochemical phenotype, localization and clinical manifestations. Amyloid β accumulates in the neuropil or within the walls of cerebral vessels, and associates with dementia or stroke, both hereditary and sporadic. Amyloid β is normally released by cells as soluble monomeric-dimeric species yet, under pathological conditions, it self-aggregates as soluble oligomers or insoluble fibrils that may be toxic to neurons and vascular cells. Lowering amyloid β levels may be achieved by inhibiting its generation from the amyloid β-precursor protein or by promoting its clearence by transport or degradation. We will summarize recent findings on brain proteases capable of degrading amyloid β with a special focus on those enzymes for which there is genetic, transgenic or biochemical evidence suggesting that they may participate in the proteolysis of amyloid β in vivo. We will also put in perspective their possible utilization as therapeutic agents in amyloid β diseases.

KEY WORDS: Amyloid β; neurodegeneration; brain proteases; therapy; cerebrovascular amyloidoses; Alzheimer disease.

INTRODUCTION

The deposition of amyloid β peptide (Aβ) in the brain is a central process in several human neurodegenerative disorders that we have proposed to group as "amyloid β diseases" (AβD)(1) (Table I, ref. 2–33). In Alzheimer's disease (AD) and Down syndrome, Aβ is mainly found in the neuropil within senile plaques, while in sporadic and hereditary amyloid angiopathies, Aβ accumulates predominantly in cortical and leptomeningeal vessels leading to stroke or multi-infarct cognitive deterioration (Fig. 1). To a lesser extent, similar cerebral Aβ deposits are also found in normal aging (34). Aβ is generated as a soluble 4-kDa peptide by internal proteolysis of a transmembrane amyloid β precursor protein (AβPP) (35,36). AβPP is cleaved sequentially by BACE1, a novel transmembrane aspartyl protease ("β-secretase") that cuts at the amino-terminus of Aβ, generating a 99-residue carboxyl-terminal stub of AβPP that is the substrate for the γ-secretase complex which, in turn, generates Aβs of different carboxyl-termini. An alternative pathway of AβPP processing by metalloendoproteases of the TACE/ADAM family ("α-secretase") cleaves between Aβ positions 16 and 17 thus preventing the release of intact Aβ by γ-secretase (reviewed in 37). Although Aβ is highly heterogeneous both at its amino and carboxyl ends, minor isoforms ending at Ala42 and Thr43 are believed to play a major pathogenic role in AD. Several lines of evidence have favored such hypothesis: (i)

* Special Issue in honor of Dr. Juana Maria Pasquini.

[1] Instituto de Química y Fisicoquímica Biológicas (IQUIFIB). CONICET. Cátedra de Química Biológica Patológica, Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Argentina.

[2] Address reprint requests to: Eduardo M. Castaño, M.D., IQUIFIB-Cátedra de Química Biológica Patológica, Departamento de Química Biológica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires. Junín 956 CP 1113, Buenos Aires, Argentina. Tel: 54 11 4 964-8288; Fax: 54 11 4 962-5457; E-mail:edcast@ffyb.uba.ar

0364-3190/02/1100–1387/0

Table I. Amyloid β Diseases

			Proposed biochemical phenotype (Aβ)				
			Increased production				
	Disease	Genetic association	Aβ total	Aβ 42/43	Aggregation increased	Decreased clearance	Ref
Sporadic	Alzheimer's disease	*ApoE, *A2M, *CysC,IDE[?],LRP[?],ACE[?]			+	++	2–13
	Cerebral amyloid angiopathy	*ApoE			+	?	14,15
	Dementia pugilistica	*ApoE				?	16,17
	Down syndrome	• AβPP	++				18
Familial (autosomal dominant)	Alzheimer's disease	Presemilin 1-2 (more than 80 mutations)		++			19
		AβPP mutations: 665	++				20,21
		670/671	++				22
		713/715		++			23
		717		++			24
		723		++			25
	Hereditary cerebral hemorrhage with amyloidosis,	AβPP 673 Aβ variant A2T			++		26
	Dutch	693 E22Q			++		27
	Italian	693 E22K				?	28
	Dementia with cerebral amyloid angiopathy:						
	Flemish	AβPP 692 Aβ variant A21G	++			+	29–31
	Arctic	693 E22G			++		32
	Iowa	694 D23N			++		33

Note: (*) Genetic polymorphisms, (•) Extra copy of AβPP gene, (?) Not confirmed/unclear.

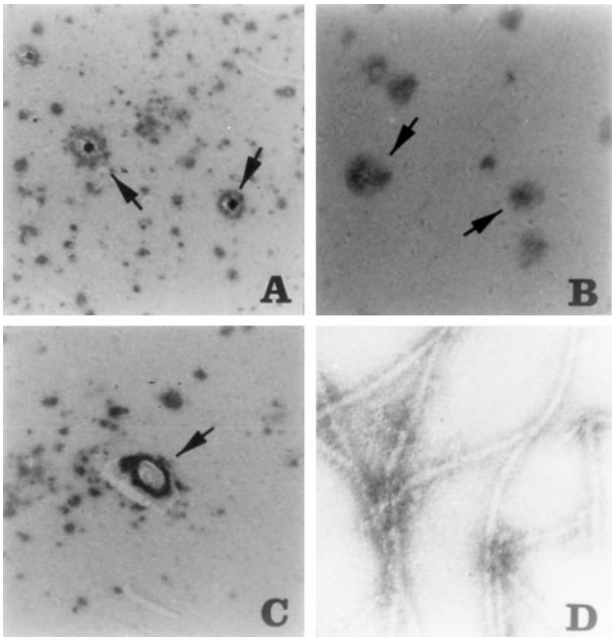

Fig. 1. Different types of Aβ deposits in AβD. Arrows indicate in (**A**) mature amyloid plaques in AD brain immunostained with monoclonal antibody 6E10 that recognizes region 1-16 of Aβ, (**B**) diffuse Aβ deposits in the brain of a Down syndrome patient labeled with antibody S42, specific for Aβ ending at Ala42 and (**C**) cortical vessels loaded with amyloid in a case of HCHWA-Dutch detected with antibody S40, specific for Aβ ending at Val40. (**D**) Electron microscopy of amyloid fibrils made of synthetic Aβ1-40 containing the Dutch substitution Glu22Gln. Original magnifications: **A**) 40×, **B** and **C**) 100×, and **D**) 100,000×.

Aβ42–43 is the major component of seniles plaques (38,39), (ii) Aβ42–43 has a lower critical concentration for amyloid formation in vitro as compared to the more abundant soluble form, Aβ40 (40), (iii) many mutations in the AβPP and presenilin genes associated with autosomal dominant forms of AD increase the steady-state ratio of Aβ42–43/Aβ40 in cultured cells and brains of transgenic mice carrying such mutants, (19,20) and (iv) Aβ42–43 is highly toxic for neurons in culture either in fibrillar or non-fibrillar oligomeric forms (reviewed in 41). In the case of vascular AβD, different pathogenic mechanisms have been proposed since the major Aβ isoform found in vascular deposits ends at Val40, with a variable degree of carboxyl-terminal truncation (42). Interestingly, in hereditary forms of amyloid angiopathy such as the Dutch or Arctic variants, Aβ contains amino acid substitutions that result in a "hyper-amyloidogenic" Aβ40 (27,32). In addition, toxicity of Aβ variants to endothelial and smooth muscle cells and disruption of the equilibrium between coagulation and fibrinolysis may contribute to the striking vascular phenotype of these rare diseases (43). Regardless of the mechanism, and within the frame of the hypothesis that Aβ is pathogenically causative in AβD, lowering Aβ levels in the brain has been proposed as a major therapeutic aim to prevent, delay or revert its deleterous effects. There are several ways to achieve such goal including: 1) to block the release of Aβ from its precursor by inhibiting β and/or γ-secretases, 2) to promote the α-secretase cleavage of AβPP, 3) to enhance the degradation of AβPP stubs before they are attacked by the γ-secretase complex and 4) to promote Aβ clearence by transport or degration (Fig. 2). The first strategy is being intensively pursued with the recent development of potent transition-state analog inhibitors (37). Several cellular factors have been described that shift the internal proteolysis of AβPP towards the non-amyloidogenic pathway including depletion of cholesterol, activation of PKC and estrogen signaling (44–46). The third possibility is based on the finding that the levels of AβPP carboxyl-terminal β stubs seem to be rate-limiting for Aβ generation (47). As a contribution to the fourth strategy, here we will summarize the recent progress on proteases that degrade Aβ, with a special emphasis on those proteinases for which there is genetic, transgenic or biochemical evidence supporting that they may participate in the clearance of Aβ in vivo.

PROTEINASES THAT DEGRADE AMYLOID β

Neprilysin

Neprilysin, also known as neutral endopeptidase (NEP), EC 3.4.24.11, enkephalinase, CD10, and common acute lymphoblastic leukemia antigen (CALLA), is a typical zinc metallopeptidase with the HExxH zinc binding motif and a consensus sequence ExxA/GD in which the glutamate serves as the third zinc ligand. NEP is potently inhibited by phosphoramidon and thiorphan, a property shared with DINE, PEX and the recently cloned NEPLP α/β among the members of the M13 family of endopeptidases (reviewed in 48). NEP is a type II integral membrane protein of ca. 700 residues with a short amino-terminal domain facing the cytoplasm and a large lumenal domain that contains the active site. NEP localization at the cell surface allows it to hydrolyze peptides on the extracellular side of the plasma membrane including enkephalins, substance P, atrial natriuretic peptide, somatostatin, endothelin and insulin B chain. Its possible physiological role is broad, and comprises the regulation of natriuretic and vasodilator peptides in the kidney, the modulation of inflammatory response by neutrophils and the inactivation of mitogenic signaling in various cell types (reviewed

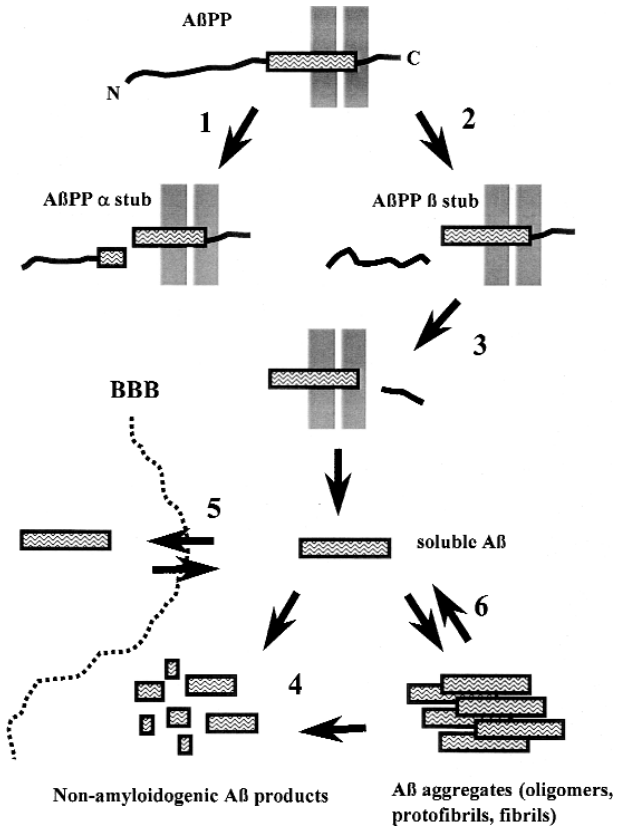

Fig. 2. Schematic pathway of Aβ metabolism in the brain and potential therapeutic targets to lower its concentration. Transmembrane AβPP is cleaved at the lumenal or extracellular side by α-secretase, a metalloprotease of the ADAM/TACE family (**1**), to yield a soluble AβPP amino-terminus and AβPP carboxyl-terminal "α stub". This non-amyloidogenic processing is modulated by several factors such as cholesterol levels, PKC, estrogen or plasmin activation. Alternatively, AβPP is cleaved at the amino-terminus of Aβ by BACE 1 (**2**) generating AβPP carboxyl-terminal "β stub" that is a substrate for the γ-secretase complex (**3**). Such processing yields Aβ of different lengths released from the membrane as soluble products. Note that γ-secretase cleavage of AβPP "α stub" was not depicted. Targeting β and γ-secretases with specific inhibitors may prevent Aβ generation. Soluble Aβ is thought to be cleared by intracellular and extracellular proteolysis (**4**) and reverse transport through the blood brain barrier (BBB) (**5**). Under pathological conditions, Aβ undergoes a transition from soluble non-toxic species to neurotoxic aggregates in the form of oligomers, protofibrils and amyloid fibrils (**6**) that may resist further proteolysis. Promoting the degradation of soluble or aggregated Aβ may prevent, retard or revert the formation of toxic Aβ.

in 49). In the brain, NEP predominates in the neuropil and is located at the synapses where it may play an important role in terminating the action of several neuropeptides (50). An early report by Sato et al. showed that a monoclonal antibody against NEP immunostained senile plaques in AD brain with a pattern and intensity similar to those obtained with anti-Aβ antibodies (51). Howell et al. showed that NEP was able to hydrolyze synthetic Aβ1-40 in vitro and their HPLC-mass spectrometry analysis revealed major sites of cleavage at Glu3-Phe4, Gly9-Tyr10, Phe19-Phe20, Ala30-Ileu31 and Gly33-Leu34 (52). More recently, Iwata et al. reported that synthetic radiolabeled Aβ1-42 injected into rat hippocampus was degraded by a NEP-like activity. Moreover, a catabolic intermediate, Aβ 10-37, consistent with the specificity of NEP, was identified, and the continuous infusion of thiorphan resulted in the accumulation of endogenous Aβ (53). In mice in which the NEP gene had been disrupted, the capability of degrading exogenous Aβ1-42 and endogenous Aβ40/42 was impaired in a gene-dose dependent manner, interestingly, endogenous Aβ accretion in this animal model was highest in hippocampus and lowest in cerebellum, along with the severity of pathology in AD (54). Studies in post-mortem human brain have shown that NEP mRNA and protein levels were lower in the hippocampus and temporal cortex as compared to other less vulnerable areas to plaque development or peripheral organs. Furthermore, such levels seemed to be specifically reduced in AD brains when matched with controls (55). Together, these data strongly support the hypothesis that NEP is involved in the normal degradative pathway of Aβ in the brain and that a reduction of its activity may have important pathogenic implicances in AD. It remains to be determined if NEP also influences the fate of Aβ in the walls of cortical and leptomeningeal vessels affected in the sporadic and hereditary amyloid angiopathies.

Two proteases related to NEP have been recently described as capable of degrading Aβ. Endothelin converting enzyme-1 (ECE-1) hydrolyzed endogenous as well as synthetic Aβ in H4 neuroglioma cells and ECE-1-transfected CHO cells. Moreover, incubation of a recombinant soluble form of ECE-1 with Aβ1-40 and Aβ1-42 yielded several products including Aβ1-16, 1-17 and 1-19 as determined by mass spectrometry (56). An intronic polymorphism of angiotensin converting enzyme (ACE) has been associated with AD as a possible susceptibility genetic factor (10,11). Interestingly, Hu et al. have recently shown that ACE purified from human seminal fluid by affinity chromatography was able to cleave Aβ1-40 at Asp7-Ser8 to such extent that co-incubation of the peptide with ACE resulted in the reduction of Aβ fibrillogenesis and cellular toxicity in vitro (57).

Insulin Degrading Enzyme

Insulin degrading enzyme (IDE), also named insulysin and insulinase, is a 110 kDa neutral thiol metalloendopeptidase with an inverted zinc binding site-

HxxEH- involved in catalysis and thus belonging to the "inverzincins" superclass of metalloproteases (58,59). Although IDE is mainly cytosolic, it contains a peroxisomal-targeting sequence and it has been found associated with membranes and in cell culture conditioned medium and human cerebrospinal fluid (60). IDE has been highly conserved in evolution, it shows ubiquitous expression with preference for testis, tongue and brain, and its transcription strongly depends on the developmental stage (61,62). Regarding its physiological role, IDE has been implicated in cellular growth and differentiation, the modulation of proteasomal activity and steroid signaling; some of these functions are likely mediated by IDE's major role in the proteolytic inactivation of insulin and other growth factors (reviewed in 63). IDE is known to cleave several peptides capable of forming amyloid fibrils in vitro and in vivo including insulin, glucagon, amylin, atrial natriuretic peptide, calcitonin and Aβ (reviewed in 64). Although IDE shows some preference for basic or hydrophobic residues on the carboxyl side of the scissile bonds, there is no sequence specificity, suggesting a recognition of secondary or tertiary structure shared by amyloid-forming peptides (65). Moreover, it has been proposed that specificity is determined at the stage of substrate binding rather than at catalysis based on the dissociation of these process after chelation or mutation of the zinc-binding site (64). Kurochkin and Goto first reported that synthetic Aβ1-28 and 1-40 were degraded in vitro by IDE purified from rat liver and that iodinated Aβs specifically cross-linked to IDE in rat brain or liver cytosolic fractions (66). A subsequent study by McDermott and Gibson showed that Aβ1-40 was effectively degraded by soluble fractions obtained from rat and human brain. The inhibitor profile and immunodepletion with a specific monoclonal antibody pointed to IDE as the main Aβ-degrading activity at neutral pH (67). These studies were extended in our laboratory to Aβ1-42 and the Dutch variant Aβ1-40E22Q at physiological concentrations, with the interesting observation that in AD brains, IDE-related Aβ degradation and IDE immunoreactivity in soluble fractions were significantly reduced as compared to age-matched controls (7). Other convergent lines of evidence have come from a series of studies on Aβ degradation in cell lines and primary cell cultures. In microglial cells, PC12 and rat cortical neurons, synthetic and endogenous Aβ were degraded by a metalloprotease with the characteristics of IDE, presumably located on the cell surface or released to the medium (60,68). Moreover, overexpression of human IDE in CHO cells led to a decrease in the intracellular and extracellular steady-state levels of endogenous Aβ, and treatment of N2a cells with insulin increased Aβ extracellular levels (68–70). Recombinant IDE was shown to cleave Aβ at His13-His14, His14-Gln15 and Phe19-Phe20 followed by Lys28-Gly29, Val18-Phe19 and Phe20-Ala21 (71). Importantly, such Aβ products were not toxic to cultured neurons and co-incubation of IDE with Aβ1-40/42 resulted in a substantial reduction in the neurotoxicity induced by these peptides. Although recombinant IDE was not able to degrade preaggregated Aβ from a "synthetic amyloid plaque (71)," it remains to be studied further whether the enzyme is able to hydrolyze soluble Aβ oligomeric species that may be neurotoxic in vivo. Genetic data have provided additional support for a possible role of IDE in AD pathogenesis since several groups reported the linkage of late-onset AD families with genetic markers near the IDE gene (4–6). Notably, in one of these studies, Aβ plasma levels were used as a surrogate quantitative trait for linkage, suggesting that this locus may increase the risk for AD by increasing Aβ levels (5). In a more recent report, however, no polymorphisms in the IDE gene have been found associated with late-onset AD and the identity of such locus remains to be determined (72).

Plasmin and Other Serine Proteinases

The plasmin system includes plasminogen/plasmin, tissue plasminogen activator (tPA), urokinase-type plasminogen activator (uPA), α2-antiplasmin (α2-AP) and plasminogen activator inhibitor (PAI-1), and has been classically involved in fibrinolysis and cell migration (reviewed in 73). tPA and uPA are serine proteinases that catalyze the conversion of the inactive plasminogen into plasmin, another serine protease, in a process that takes place on the cell surface and involves the interaction with matrix metalloproteases (reviewed in 74). In the brain, tPA, uPA and plasminogen are synthesized by neurons, plasmin is abundant in the hippocampus and tPA is produced by microglia (75). The initial evidence of a relationship between Aβ and the plasmin system came from the finding that fibrin and Aβ showed cross-reactivity with the respective antibodies, suggesting structural similarities. Moreover, aggregated Aβ was able to stimulate the activity of tPA in vitro (76,77). Van Nostrand and Porter reported that plasmin was able to cleave Aβ1-40 at Arg5-His6 generating an Aβ 6-40 species with high β-sheet content that, in turn, strongly stimulated tPA (78). In vitro degradation of Aβ1-40 by plasmin was further studied by Tucker et al., who showed that plasmin cleaved the peptide at all the predicted sites within the Aβ se-

quence in a concentration-dependent manner. Moreover, plasmin was able to degrade fibrilar Aβ1-40, although with a 20-fold reduction in Kcat as compared with nonfibrilar Aβ. Consistent with these results, plasmin protected rat cortical neurons from Aβ toxicity presumably through direct degradation of the peptide (79). The induction of tPA and uPA mRNAs in cells treated with aggregated Aβ and the increase of tPA and uPA mRNAs in the brains of AβPP transgenic mice suggest that the plasmin system may be activated in response to Aβ deposition, a mechanism that correlates with the reported up-regulation of PAI-1 in AD (80). Yet, a recent paper by Ledesma et al. showed low levels of plasminogen and plasmin in the neocortex and hippocampus of AD brains raising the possibility that up-regulation of the plasmin system by Aβ becomes exhausted in the course of the disease, perhaps associated with progressive neuronal loss. Interestingly, activation of plasmin localized in membrane rafts of cultured hippocampal neurons reduced Aβ levels by the promotion of the cleavage at the α-secretase site of AβPP in addition to Aβ degradation (81).

An unidentified 28 kDa serine protease from fetal calf serum or pancreatic trypsin preparations that formed a 700 kDa stable complex with α2-macroglubilin (A2M) was described as capable of degrading soluble Aβ1-40/42 (82). More recently, human neuroblastoma cells in which the expression of metalloendopeptidase EC 3.4.24.15 (MP24.15) was lowered by antisense transfection showed a marked reduction in Aβ degradation. Recombinant MP24.15 did not degrade Aβ directly, therefore such effect was attributed to the activation of a 28 kDa serine-protease complexed with α1-anti-chymotrypsin (ACT), a protease inhibitor that binds Aβ and is present in senile plaques (83–85). Although the role of these unidentified serine proteases in the brain is unknown, it is noteworthy that MP24.15 expression declines with age in the temporal cortex and its levels are severely reduced in AD brains (86).

Matrix Metalloproteinases

Matrix metalloproteinases (MMPs) are a family of at least 25 members involved in degradation and remodeling of extracellular matrix. They have common propeptide and N-terminal catalytic domains, while the presence of transmembrane domains, fibronectin-like repeats and carboxyl-terminal hemopexin-like domains allow categorization of MMPs into the collagenase, gelatinase, stromelysin and membrane-type MMP subfamilies (reviewed in 87). MMPs are secreted in a latent form that requires a proteolytic processing step for activation and their activity is regulated by tissue inhibitors (TIMP) which bind to latent and active forms of the enzyme (reviewed in 88). Interestingly, TIMP have been found by immunohistochemistry to co-localize with neuritic plaques and neurofibrillary tangles in AD (89). MMP-9 (EC 3.4.24.35) has been detected in pyramidal neurons of normal human hippocampus and appears to be overexpressed in AD brains as compared to controls. Moreover, in AD, MMP-9 localized to neuritic plaques and a latent form of the enzyme was shifted to an insoluble compartment (90). When purified MMP-9 was incubated with synthetic Aβ1-40, several products of degradation were found with a major site of cleavage at Leu34-Met35 after short incubation times followed by Lys16-17, Ala30-Ileu31, and Gly37-38 (90). Notably, Aβ species ending at Gly37 have been identified in amyloid extracted from leptomeningeal and cortical vessels in HCHWA-D consistent with MMP-9 or NEP activity (42). MMP-2 is activated to 64–66 kDa isoforms by a plasma membrane dependent mechanism that involves the activity of membrane-type MMPs (91). Active forms of recombinant MMP-2 can degrade Aβ1-40 and 1-42 purified from vascular amyloid with major hydrolysis at Lys16-Leu17, Leu34-Met35 and Met35-Val36 (92). Moreover, treatment of primary hippocampal cultures with Aβ1-40 increased the production of MMP-2, MMP-3 and MMP-9 while treatment of human glioblastoma cells with Aβ1-40 and 1-42 resulted in a selective induction of MMP-2 activity, possibly mediated by increased expression of membrane type-MMPs (93,94).

Endosomal/Lysosomal Proteases

Numerous studies using a variety of cultured cells have supported the notion that cellular internalization is a major pathway for the clearence of extracellular Aβ. Various mechanisms have been described, including nonsaturable Aβ uptake as well as receptor-mediated endocytosis by the serpin-enzyme complex, LDL-receptor related pathway and scavenger receptor (95–98). Among these, considerable attention has been given to the LDL receptor-related protein (LRP) pathway that mediates the internalization of several ligands involved in lipoprotein metabolism and protease/protease inhibitor complexes (reviewed in 99). LRP ligands include apolipoprotein E, α2-macroglobulin (A2M) and isoforms of AβPP containing the Kunitz protease inhibitor domain, all genetically associated with AD (reviewed in 100). Moreover, LRP expression in transgenic mice may be influenced by presenilin pathogenic mutations (101). LRP are highly expressed in the brain, with LPR-1 predominating in the neuropil and LRP-2 (also known as gp330, brushin and megalin) being expressed by

choroid plexus and ependymal cells, in contact with cerebrospinal fluid (CSF) (102,103). While LRP-1 internalizes Aβ bound to A2M and lactoferrin, LRP-2 mediates cellular clearance of Aβ complexed to apolipoprotein J (103). As a result of cellular internalization, Aβ is thought to be degraded by lysosomal proteases including cathepsins D, B and S which have been shown to proteolyze Aβ in vitro (31,104–107). Moreover, treatment of cultured cells with chloroquine inhibited the degradation of Aβ internalized as a complex with apo J, and coinfusion of Aβ1-40 with leupeptin in rat brain resulted in extracellular and intracellular Aβ accumulation, consistent with a role of lysosomal proteases in Aβ clearence (103,108). Interestingly, several changes have been described in the lysosomal system in AD, including the accumulation of lysosomes and lysosomal hydrolases inside vulnerable neurons and extracellularly, in close association with Aβ deposits (109,110). Although cathepsin D protein levels and activity are known to increase with normal aging in the brain (111–113), a specific increase of cathepsin D in the CSF of AD patients and the induction of cathepsin D by Aβ in human hippocampal slices have been reported (114,115). Recently, a polymorphism in the cathepsin D gene has been associated with sporadic AD (116).

STRATEGIES FOR INCREASING Aβ DEGRADATION IN THE BRAIN

Targeting Endogenous Inhibitors

Several endogenous inhibitors have been described that may regulate the activity of Aβ-degrading proteases. A heptapeptide with the sequence LVVYPWT that inhibits NEP has been isolated from bovine spinal chord (117), and a heat-stable 14 kDa component that co-purifies with IDE from the liver has been postulated as an endogenous inhibitor of this enzyme (118,119). Some AβPP isoforms contain a Kunitz-type inhibitor of serine proteinases and all isoforms include a domain capable of inhibiting MMP2 (120–122). Yet, the role that these inhibitors may have in the regulation of Aβ proteolytic pathway in the brain is not known. ACT, as mentioned above, is associated with AD lesions and capable of forming detergent-resistant complexes with Aβ. Recently, it has been shown that ACT/AβPP double transgenic mice have a significant increase in amyloid deposition and soluble Aβ levels as compared to mice that overexpress AβPP only, suggesting that in addition to a possible role in promoting Aβ aggregation, ACT may inhibit its clearence by serine-proteases (123, 124). Furthermore, the A allele of ACT, which has been suggested to increase the risk for AD, may enhance the levels of secreted ACT in cultured astrocytes (125). Cystatin C (cysC), a secreted lysosomal cysteine protease inhibitor has been found associated with amyloid deposits in AD and HCHWA-D and it is increased in susceptible neurons in AD (126–128). Moreover, a polymorphism in the cysC gene has been recently associated with an increased risk for late-onset AD and it remains to be addressed whether such polymorphism affects Aβ proteolytic turnover (12,13). These examples underscore that the balance between Aβ-degrading proteases and their endogenous inhibitors may be altered in AβD and therefore, decreasing their levels or activity is a potential way of restoring or enhancing Aβ degradation.

Promoting the Expression and Activation of Proteases

Transcriptional up-regulation has been proposed as a mechanism of increasing NEP activity in the brain (54). The expression of NEP is regulated in a tissue-specific manner and several NEP mRNAs with different 5′ UTR have been described. Type I NEP mRNA, that includes exon 1, has been identified as the major transcript in neurons (129). At least one enhancer region and several clusters of transcription factors binding sites have been found upstream of exon 1 (130). Moreover, expression of NEP is modulated by steroids through the interaction with specific elements, one that binds androgen, progesterone and glucocorticoid receptors in the 3′ UTR and a unique androgen responsive sequence in the promoter region (131). Although IDE expression is known to be developmentally regulated, very little is known about the factors that modulate its transcription. In human neuroblastoma cell lines, IDE expression is significantly increased by retinoic acid and synthetic retinoid agonists that bind to RAR α (132). Activation of latent precursors is an alternative strategy in the cases of MMPs or plasminogen, although there are evidences that these proteases, as well as the lysosomal system, may be chronically activated in some AβD such as AD. Yet, recent results in transgenic mice that overproduce Aβ suggest that intracellular degradation of the peptide—both soluble and aggregated—by brain phagocytic cells may be further stimulated by anti-Aβ immunoglobulin treatments (133).

Delivery of Recombinant Proteases

The delivery of recombinant proteases represents, in theory, a direct way of increasing Aβ-degrading activity in the brain and its vasculature. The systemic ad-

ministration of biologically active recombinant proteins to the CNS using different strategies to permeabilize the blood brain barrier has been described (134, reviewed in 135). However, a localized and regulated delivery may be preferable to achieve a therapeutic degradation of Aβ restricted to vulnerable regions in the brain. In this respect, several strategies are being investigated, including the transplantation of cells, either naked or encapsulated within polymer membranes with selective permeability, that overexpress and release the protein of interest, (reviewed in 136). Viral vectors are becoming increasingly important for transgene delivery and protein expression in the CNS. Among these, vectors with large cloning capacity, high transduction efficiency and sustained expression such as replication-defective lentiviral vectors may be suitable for the localized delivery of recombinant proteases (137). The development of vectors containing elements such as the tetracycline-inducible system may be useful to overcome the problem of continuous expression of proteases and its potential side effects (138). Yet, several safety issues have to be addressed before these technologies can be widely used in humans, including the detection of competent recombinant particles or germ line transmission. Moreover, direct delivery of recombinant proteins, cells or vectors into the CNS requires invasive procedures that may impose additional risks.

REMAINING QUESTIONS AND PERSPECTIVE

As the identification and characterization of brain proteases capable of degrading Aβ progress, several aspects of Aβ metabolism remain to be clarified, both in normal conditions and disease. It is not yet clear which is the main compartment for the Aβ degradative process in the brain and whether the initial Aβ oligomerization in AβD takes place intra or extracellularly. In this regard, it is noteworthy that Aβ is generated intracellularly within secretory compartments and that aggregation of Aβ42 inside the cell may be highly toxic to human neurons (139,140). Although the putative major Aβ-degrading proteases span extracellular and intracellular locations, it may be necessary to study the relative weight of each of them on Aβ clearance by studying conditional knockout mice, and to assess the effect of the lack of specific proteases upon Aβ accretion at the subcellular level. In this regard, the only informative animal model reported so far has been the NEP knockout mouse(54). The apparent redundancy in Aβ proteolysis (including several unrelated proteases that cut bonds that may be important for amyloid formation such as the Phe19-Phe20 or Gly37-Gly38), suggests that the process is ubiquitous with Aβ degradation taking place, at a given time, in different compartments and by different proteases. However, in the case of AβD due to Aβ genetic variants, protease specificity or affinity may be shifted by the primary structure of the peptide or its conformation. Moreover, accessibility of proteases to Aβ may be influenced by the site of Aβ accumulation (i.e., vessel wall vs neuropil). As a remarkable example, the Flemish variant of Aβ Ala21Gly is inefficiently cleaved at Phe19-Phe20 by cathepsin D while the Dutch variant Aβ Glu22Gln is hydrolyzed at the same rate as the wild type (31). In this respect, it is also important to emphasize that a series of post-translational modifications have been found in Aβ isolated from AD brains including isomerization, racemization, cyclization and oxidation, while the activity of proteases upon these Aβ species is not known (141–143). The wide variety of peptides that are substrates of the proteases capable of degrading Aβ raises the concern of possible side effects of this therapeutic strategy. The matter has to be carefully addressed in animal models to evaluate the impact that protease manipulation may have on basic aspects of brain physiology such as insulin signaling, neuropeptide transmission, regional blood flow, clot formation or extracellular matrix integrity. In this regard, fibrinolytic therapy of myocardial infarction with tPA has been associated with cerebral hemorrhage in patients with cerebral amyloid angiopathy (144), therefore, pharmacological activation of the plasmin system may be particularly problematic in this group of patients. Several proteolytic systems seem to be chronically stimulated in AD brains, including lysosomal proteases, tPA/plasminogen/plasmin and MMPs and therefore, increasing their expression or activity may have little consequence on Aβ metabolism. On the other hand, the promotion of the expression or activity of proteases that are thought to be decreased in AD such as NEP or IDE may have profound effects on the steady-state levels of soluble Aβ. Yet, once Aβ has begun its aggregation process, other strategies such as the enhancement of phagocyte-mediated clearence may be more effective in the proteolytic removal of the peptide. With the transgenic animal models currently available, the proteases that degrade Aβ can be modulated and the consequences upon Aβ levels and its purported toxicity assessed in vivo. These experiments may be decisive to clarify the potential of treatments based on Aβ degradation for the management of AβD. A combination of therapies that effectively lower Aβ in the brains of AβD patients represents the ultimate test for the amyloid hypothesis and, hopefully, a long-sought step forward in the treatment of these devastating conditions.

ACKNOWLEDGMENTS

We dedicate this work to Giannina Pasquini. We thank Mikio Shoji and Etsuro Matsubara for S40 and S42 antibodies. This work was supported by Agencia Nacional de Promoción Científica y Tecnológica, Argentina (ANPCyT Grant PICT98-05-04394) and Ministerio de Salud de la Nación (Becas Carrillo-Oñativia 2001 to LM and EMC).

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