1044-7393/96/2703—0225 $10.75

The Possible Place of Cathepsins and Cystatins in the Puzzle of Alzheimer Disease

A Review

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Received July 18, 1995; Accepted July 20, 1995

ABSTRACT

Lysosomal proteinases (cathepsins) and their endogenous inhibitors (cystatins) have been found to be closely associated with senile plaques, cerebrovascular amyloid deposits, and neurofibrillary tangles in Alzheimer disease (AD). Further, profound changes in the lysosomal system seem to be an early event in "at-risk" neurons of AD brains. There is an ongoing controversy as to whether lysosome-associated proteolytic mechanisms are causally related to the development and/or further progression of the disease. The present article deals with some arguments "pro" and "contra" an involvement of the endosomal/lysosomal pathway in amyloidogenesis as a cardinal process in AD. Other putative targets of acidic proteinases and their natural inhibitors in the pathogenesis of AD (such as formation of neurofibrillary tangles and regulation of apolipoprotein E) are also discussed.

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Index Entries: Cathepsins; cystatins; lysosomes; immunohistochemistry; Alzheimer disease; amyloidogenesis; proteolysis; neurofibrillary tangles; apolipoprotein E.

INTRODUCTION

Alzheimer disease (AD) is a progressive degenerative encephalopathy that effects a considerable portion of the elderly human population. AD is clinically characterized by profound behavioral disorders, loss of memory and reasoning, and personality changes. Neuropathologic hallmarks of AD are accelerated atrophy and loss of neurons from specific areas of the brain, reduction of synapses on surviving neurons, deposition of amyloid in neuritic plaques and within the wall of the cerebral microvasculature, and the increased appearance of neurofibrillary tangles (for overview, *see* Masliah and Terry, 1993; Sisodia and Price, 1993; Solkoe, 1994). Although some of these pathologic changes may occasionally be observed in brains of aged patients without clinical signs of AD, there is no doubt that these hallmarks are highly indicative of AD, and that the degree of their expression correlates with the severity of the disease. In search of the mechanisms underlying these degenerative alterations, proteolytic enzymes have been shown to play important roles. Though one hardly can find a brain-associated proteinase that never has been suspected of being involved in AD, recent research has concentrated on a dozen of the most interesting candidates. These include the calpains I and II, the multicatalytic proteinase, some metalloproteinases, plasminogen activator, and the cathepsins B, D, E, G, H, L, and S (for references, *see* Bernstein and Wiederanders, 1994). Cathepsins are—with the exception of cathepsin E—lysosome-located, acidic proteinases that account for a considerable portion of the overall protein turnover and possibly for the processing of certain neuropeptides in the CNS. They moved into the field of view of Alzheimer research because several investigators had observed that cathepsin-immunoreactive material is closely associated with senile plaques and neurofibrillary tangles. However, anatomical localization of an enzyme (or any other chemical compound) within or nearby plaques and tangles alone does not prove the existence of a causal link between the substance and the lesion. The present article attempts to deal with some arguments "pro" and "contra" an involvement of lysosomal proteinases in the initiation and/or further progression of AD. Special emphasis is given to their potential role in the process of amyloid formation, but other putative targets of acidic proteinases in the pathogenesis of AD (such as formation of neurofibrillary tangles and regulation of apolipoprotein E) are also considered.

CATHEPSINS AND CYSTATINS: NOMENCLATURE AND SOME PROPERTIES

Briefly, cellular endopeptidases may be subdivided into the following families (after Barett, 1980; McDonald, 1985; Bernstein, 1994):

1. Serine proteinases (EC 3.4.21) have a serine in their active center. Organophosphorus compounds are inhibitory. A lysosomal serine proteinase is cathepsin G. Known endogenous inhibitors are α_1-antitrypsin and α_1-antichymotrypsin (Abraham et al., 1988).
2. Cysteine proteinases (EC 3.4.22) contain cysteine in their active site. This cysteine is activated in the presence of exogenous thiols and inhibited by leupeptin, E 64, and other blockers. There exist natural inhibitors of this class of proteases (cystatins A, B, C, S, AS, and SN; α_2-macroglobulins and kininogen; Abrahamson et al., 1986). To this type of proteinase belong the cathepsins B, H, L, and S.
3. Aspartic proteinases (EC 3.4.23) are known to be pepstatin-sensitive. A lysosomal aspartic proteinase is cathepsin D, whereas cathepsin E is a nonlysosomal representative of this group.
4. Metalloproteinases (EC 3.4.24) contain a metal in their catalytic center. They are active at pH 7.0–9.0 and EDTA-sensitive. An example of these enzymes is collagenase.

THE FORMATION OF AMYLOID AND CATHEPSINS

Intracerebral and cerebrovascular deposition of amyloid is a cardinal phenomenon in AD. The major chemical component of the lesion is extracellularly located amyloid fibrils composed of the so-called β-peptide. This 4-kDa peptide is generated from larger precursors (amyloid precursor proteins, APPs), which are membrane-associated glycoproteins having structural features of cell-surface receptors or growth factors (Kang et al., 1987; Whitson et al., 1989).

The APP gene is located on human chromosome 21 and consists of 18 exons, which are alternatively spliced into several different transcripts (APP isoforms), named accordingly to their length in amino acids, APP_{695}, APP_{751}, and APP_{770}. Two of them (APP_{751} and APP_{770}) have an extra sequence homologous to the Kunitz-type trypsin inhibitor (Tanzi et al., 1988, and for a comprehensive review, *see* Selkoe, 1994).

Amyloid precursor proteins are not only widely distributed within normal and AD brains, but also in nonneural tissues. β-peptide, however, seems to form amyloid only in AD and aged brains of humans and a few other higher vertebrates. The reason for this is yet not fully clear, but

possibly has to do with the species-specific amino acid composition of β-peptide (Dyrks et al., 1993) and the proteolytic processing of the APPs (Price et al., 1992; Jarrett et al., 1993). After maturation through a constitutive secretory pathway, which includes several posttranslational modifications of the molecule (Weidemann et al. 1989; Oltersdorf et al., 1989), APPs are cleaved at the plasma membrane (Sisodia et al., 1990; Haass et al., 1992a). Although a larger portion of normal secretory processing involves cleavage within the β-amyloid domain and thus precludes β-peptide generation (Weidemann et al., 1989; Sisodia, 1992; Esch et al., 1992), a certain part of the APPs is split in such a way that β-peptide and closely related peptides are secreted (Haass et al., 1992a; Seubert et al., 1992; Shoji et al., 1992; Busciglio et al., 1993; Dyrks et al., 1994). Hence, β-peptide must be regarded as a normal cell product being released from neurons and nonneuronal cells, both in normal and AD brains as well as in other tissues (Selkoe, 1994).

The results of various pharmacological, histochemical, and subcellular fractionation experiments have led to the opinion that APPs are also processed in an alternative pathway, in the endosomal-lysosomal system (Cole et al., 1989, 1992; Golde et at., 1992; Caporaso et al., 1992; Haass et al., 1992b; Kosik, 1992; Siman et al., 1993; Sun et al., 1994). For a short period of time (namely before the detection of the liberation of β-peptide from its precursor through the secretory pathway), it was a common belief that the endosomal-lysosomal way was entirely responsible for the generation of β-peptide and other potentially amyloidogenic fragments from APPs, and that the cathepsins were putative enzyme candidates to manage this. Although this hypothesis in its rigid version is not any longer valid, there are still a lot of good arguments to support the notion that the endosomes-lysosomes may contribute to the production of β-peptide and larger amyloidogenic fragments. First of all, lysosome-like morphologic structures and with them lysosomal proteinases (and other lysosomal hydrolases) accumulate in degenerating neurons and around amyloid plaques of AD patients and subjects with Down syndrome (Agostini, 1952; Suzuki and Terry, 1967; Bernstein et al., 1989, 1990, 1992, 1994b, c; Cataldo et al., 1990, 1991, 1994; Nakamura et al., 1991; Nixon et al., 1992, 1993; Ii et al., 1993; Figs. 1–4). Moreover, these plaque-associated cathepsins are enzymatically active (Cataldo and Nixon, 1990). The association between the localization of lysosomal proteinases and neuritic plaques is so stable that cathepsin immunostaining may be used as an additional diagnostic tool in AD neuropathology (Bernstein et al., 1992a; Banay-Schwartz et at., 1994; Cataldo et al., 1994; Maatschieman et al., 1994). Second, APP and APP fragments are concentrated in lysosomes (Bancher et al., 1989; Benowitz et al., 1989; Cole et al., 1989). Third, the endosomal-lysosomal pathway is responsible for APP processing into β-peptide-bearing fragments (Caporaso et al., 1992; Cole et al., 1992; Golde et al., 1992; Haass et al., 1992b; Hayashi et al., 1992; Kosik, 1992; Knops et al., 1992; Siman et al., 1993). Fourth, there is an increase in a (perhaps neurotoxic;

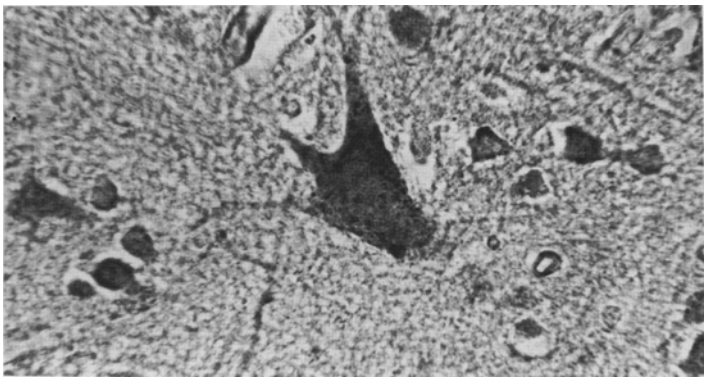

Fig. 1. Cathepsin-immunoreactive neuron in the angular gyrus of a 6-yr-old boy suffering from Down's syndrome. Note the intracellular clustering of the reaction product.

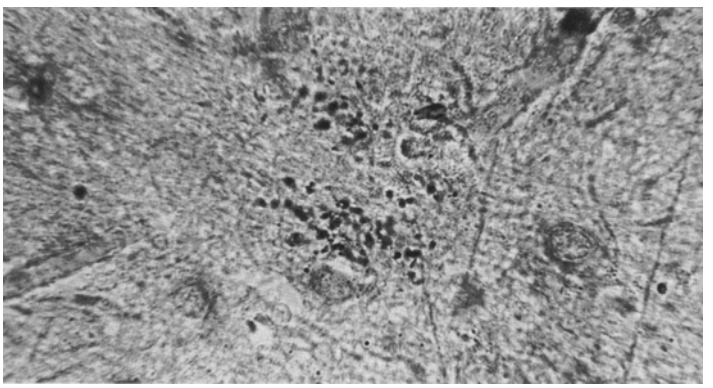

Fig. 2. Extracellularly located cathepsin B immunoreactivity: lysosome-like structures in a diffuse plaque. The same brain area and subject as in Fig. 1.

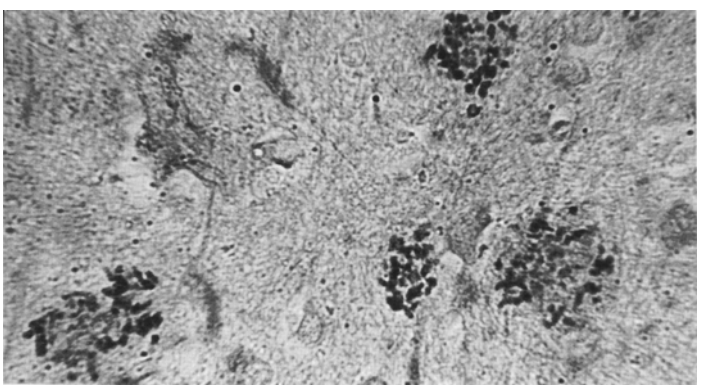

Fig. 3. Cathepsin S immunoreactive senile plaques in the prefrontal cortex from an AD brain.

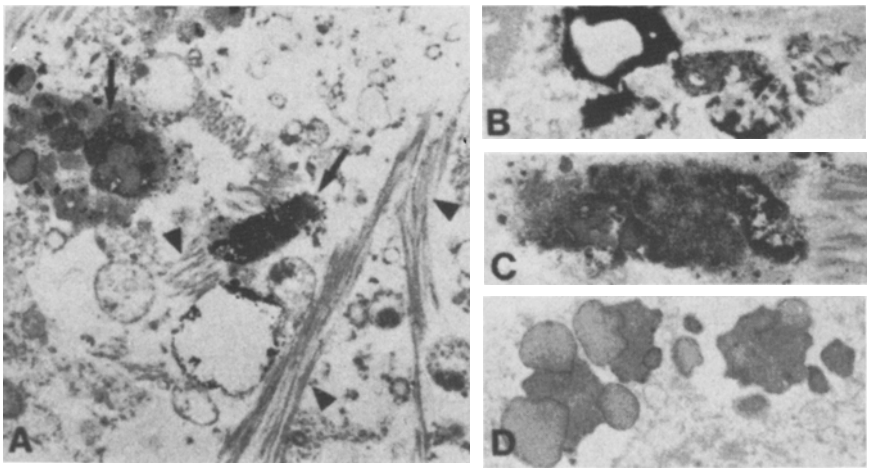

Fig. 4. Ultrastructural localization of cathepsin D in senile plaques. Immunoreactive material was associated with lipofuscin granules and amyloid fibrils (A–C). Lipofuscin granules from unstained tissue sections (D) served as negative controls for cytochemical preparations. From Cataldo and Nixon (1990), with courtesy of the authors.

Yankner et al., 1989) C-terminal fragment with human aging (Norstedt et al., 1991), which is possibly identical with one of the COOH-terminal fragments found within endosomes-lysosomes. Fifth, the acidic pH level of the lysosome strongly promotes the aggregation of β-peptide to amyloid (Burdick et al., 1992; Knauer et al., 1992). This is of special importance, because the production of β-peptide *per se* is possibly not pathogenic (though there are reports on neurotoxic actions of APP fragments and of β-peptide itself as reviewed by Bahr et al., 1994; Schubert et al., 1995). Sixth, cysteine proteinase inhibitors (leupeptin, E 64) and acidotrophic agents (chloroquine, ammonium chloride) induce a buildup of potentially amyloidogenic fragments in dissociated neurons (Caporaso et al., 1992, 1994; Cole et al., 1992; Wolozin et al., 1992; Gandy and Greengard, 1992; Bahr et al., 1994; Hajmohammandreza et al., 1994; Tsuzuki et al., 1994). However, the interpretation of these results is not so simple, since leupeptin is not specific for lysosomal cysteine proteinases, but also inhibits membrane-bound cysteine proteinases. Seventh, blockers of endocytosis and fusion of endosomes to primary lysosomes affect lysosomal processing of APP (Dash and Moore, 1993). Finally, characteristic alterations occur in the lysosomal system of "at-risk" neurons of AD brains and nerve cells from patients with Down syndrome before other neurodegenerative lesions become obvious (Nixon et al., 1992, 1993). It has been demonstrated that lysosomes dramatically increase in their number and begin to accumulate in the basal pole of the neuronal perikayon, thus giving rise to an enhanced, nonuniform immunostaining for different cathepsins. These alterations are an early marker in AD and Down syndrome

(Nixon et al., 1992) and may be of relevance not only for APP processing, but also for other neurogenerative changes in relation to lysosomes in AD. The triggering mechanism for these alterations is not well understood. Recently, Cataldo and coworkers (1995) have speculated that the early upregulation of the endosomal-lysosomal system in AD reflects increased autophagy and endocytosis in response to pathologic alterations of the cell membranes. Additionally, changes occurring with lysosomes during aging might play a crucial role. Neuronal lysosomes in the aged brain may become leaky and release proteolytic enzymes into the cellular environment, where they remain (partially) active, and may attack proteins normally not processed through lysosomes (Nakamura et al., 1989). In view of supposed evidence linking the endosomal-lysosomal system to the pathogenesis of amyloid formation, it is important to define specific proteolytic mechanisms within these acidic intracellular compartments that regulate the content of potentially amyloidogenic fragments (Siman et al., 1993). Let us consider first the putative role of the cysteine proteinases, cathepsins B, H, L, and S. With the exception of cathepsin H, all these cathepsins are present in human brain neurons and can be immunolocalized in neuritic plaque (Bernstein et al., 1990, 1992, 1994b; Cataldo and Nixon, 1990; Cataldo et al., 1991, 1994; Nixon et al., 1992; Ii et al., 1993; Lemere et al., 1994, 1995). The application of irreversible blockers to cysteine proteinases enhances the amount of amyloidogenic APP fragments within the cell (*see above*). However, recent research has demonstrated that these inhibitors do not alter the production of APP fragments. Instead, they prevent further degradation of these large fragments to smaller ones (Siman et al., 1993). This might mean that cysteine proteinases degrade potentially amyloidogenic peptides to smaller peptides that are not any longer amyloidogenic. Indeed, there is evidence that at least one of them, cathepsin B, is capable of splitting APP inside the β-peptide domain thus precluding the liberation of amyloidogenic fragments (Tagawa et al., 1991; Schönlein et al., 1993). It has therefore been speculated that one of the functions of cathepsin B is removed those "dangerous" peptides (Schönlein et al., 1993; Siman et al., 1993; Bernstein et al., 1994b). In contrast, cathepsin S is believed to produce β-peptides (Lemere et al., 1994; Munger et al., 1994; Petanceska and Devi, 1994). Cathepsin S has recently been shown to be upregulated in AD and Down syndrome brain (Bernstein et al., 1994b; Lemere et al., 1995). Postulating a function of cathepsin B in normal processing of APPs, we and others have looked for the presence of endogenous inhibitors in the brain of normal and AD brains. Cystatins A, B, and C were found to be widely distributed in the normal human CNS. The accumulation of a molecular variant of cystatin C is known to be the cause for cerebral amyloid angiopathy, also known as cerebral hemorrhage with amyloidosis of the Iceland type (Ghiso et al., 1986). In AD brains, a close association of these natural inhibitors to senile plaques and cerebrovascular amyloid was revealed (Bernstein et al., 1990, 1992, 1994b, c; Ii et al., 1993). Cystatins have been identified in human

brain neurons and glial cells as well in neuritic plaques of AD brains (Ii et al., 1993; Bernstein et al., 1994). Though direct evidence is lacking (Parfitt et al., 1993), it may well be that in AD brains cystatins inhibit cerebral cysteine proteinases by forming enzymatically inactive complexes with them, and thereby contribute to the accumulation of potentially amyloidogenic fragments. However, it is also imaginable that cystatins bound to cysteine proteinases protect the catalytic activity of these enzymes. This would partly explain the longevity of cathepsins in senile plaques as described by Cataldo et al. (1990), which is apparently much longer than their normal life-span in cells (Bohley and Seglen, 1992). It must be emphasized, however, that cystatins are not the only proteinase inhibitors found in plaques. Among others, plaques contain the serine proteinase inhibitors α_1-antichymotrypsin (Abraham et al., 1988; Van Norstrand et al., 1991), α_1-antitrypsin (Yoshida et al., 1991; Gollin et al., 1992), and thrombin/antithrombin III (Deschepper et al., 1991; Kalaria et al., 1993), which might also play roles in the normal and/or aberrant processing of APPs. In contrast to cysteine proteinases, aspartic proteinases seem to be responsible for the generation of amyloidogenic fragments. Cathepsin D was the first lysosomal proteinase that was demonstrated in Alzheimer lesions (Bernstein et al. 1989; Cataldo et al., 1990). There is an increased expression of cathepsin D mRNA in the nervous tissue of AD patients (Nixon et al., 1994; Cataldo et al., 1995), and elevated levels of the enzyme can be measured in the CSF of AD patients (Schwagerl et al., 1995). During the last year, vincing evidence came from several laboratories that cathepsin D produces β-amyloid-containing fragments from APP (Bausch et al., 1994; Dreyer et al., 1994; Haass and Sparks, 1994; Ladror et al., 1994). Moreover, processing of the APP by this enzyme is strongly enhanced by a familial (Swedish variant) AD mutation of APP (Bausch et al., 1994; Dreyer et al., 1994; Ladror et al., 1994). This is a unique property of cathepsin D and a strong argument in favor of a role of this proteinase in AD. Perhaps, the assumption of Siman and colleagues (1993) is correct in that cathepsin D generates amyloidogenic fragments, which are subsequently cleared by cathepsin B and other cysteine proteinases (except cathepsin S) once APP has entered the endosomal/lysosomal route. It is imaginable that this "two-step" mechanism for the degradation of APP through the endosomal-lysosomal pathway is disturbed in AD by either functional overload of the whole proteinase machinery or, more specifically, by the inhibition of cysteine proteinases (i.e., step two) by the cystatins. However, such a (reversible) block of cysteine proteinases has not yet been described for the nervous tissue of AD patients. Instead, Cataldo and Nixon (1990) could show that at least cerebral cathepsin B is enzymatically active in AD (Fig. 5). Another objection to a crucial role of cystatins in the generation of potentially amyloidogenic fragments of APP may come from the observation that cathepsin D upregulates cysteine proteinase activities in nonneural tissues by degrading cystatins (Lenarcic et al., 1988). Consequently, one might expect that the elevated cathepsin

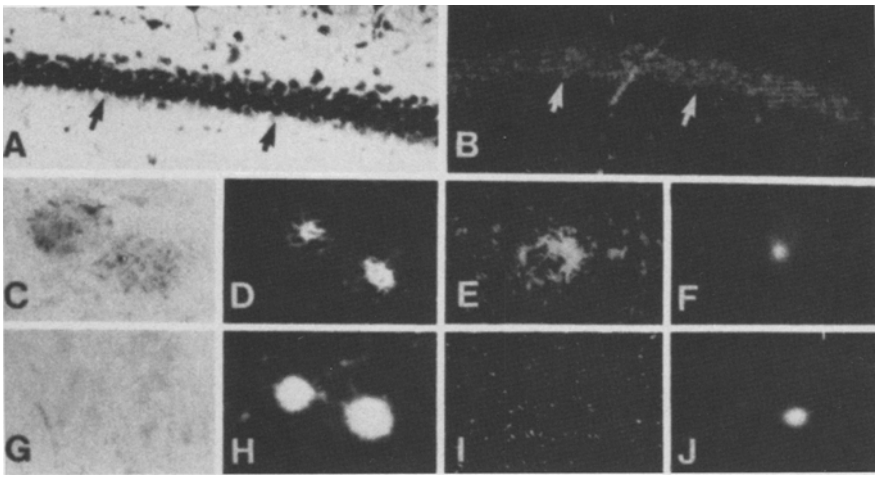

Fig. 5. Histochemical localization of cathepsin B and D activities. Pyramidal cells in hippocampal sections from mouse brain displayed high levels of cathepsin B (A, arrows) and cathepsin D (B, arrows) activities. In the prefrontal cortex of Alzheimer brains, senile plaques were confirmed in the same sections by thioflavin S-positive histofluorescence (D and F). Histochemical activities were eliminated after preincubation with leupeptin (G) and pepstatin (I). Protease-containing plaques demonstrating inhibitor sensitivity were identified by counterstaining with thioflavin S (H and J). From Cataldo and Nixon (1990), with courtesy of the authors.

D activity in AD brains (Banay-Schwartz et at., 1994; Cataldo et al., 1995) lowers the tissue content of cystatins. However, immunohistochemical findings from Bernstein et al. (1990, 1992b, 1994b) and Ii et al. (1993) clearly demonstrate an increase (instead of a decrease) in immunodetectable cystatins A, B, and C in AD brains. Perhaps, this regulatory mechanism does not work in the human nervous tissue. Another important question to be answered is under which circumstances cells use the endosomal-lysosomal way for APP processing. It has been argued that this pathway is used only in situations of an overproduction of APP functioning as an "overflow pipe" or that the use of this route results from a "missorting" of full-length APP (Kuentzel et al., 1993; Bernstein et al., 1994b).

There is recent evidence in favor of the idea that other acidic compartments of the cell (late Golgi apparatus/early endosomes, secretory vesicles, parts of the endoplasmic reticulum) may also contribute to the production of β-peptide (Dash and Moore 1993; Kuentzel et al., 1993; Norstedt et al., 1993; Koo and Squazzo 1994; Higaki et al., 1995). Therefore, cells in which some lysosomal functions are deficient owing to a genetic defect in mannose phosphorylation still generate the β-peptide (Podlisny et al., 1993). Further, pharmacological investigations employing inhibitors of Golgi processing, such as monensin and brefeldin A, were found to block the formation of β-amyloid (Busciglio et al., 1993; Haass et al., 1993), whereas short-time treatment with the lysosomotropic agents chloroquine and

NH_4Cl had only modest effects on APP processing to potentially amyloidogenic fragments (Busciglio et al., 1993; Higaki et al., 1995). APP has frequently been found in clathrin-coated vesicles, and finally, the 4-kDa β-amyloid peptide may be produced independently of the secretory pathway (LeBlanc and Gambetti, 1994). The involvement of nonlysosomal acidic compartments does not mean, however, that cathepsins may be excluded from the list of enzyme candidates to be involved in APP processing. Though their typical localization is lysosomal, cathepsins have been demonstrated at other cellular sites, too (Runquist and Havel 1991). Cathepsin B, for example, occurs in lysosomes and the endoplasmic reticulum of rat brain neurons as revealed by EM immunocytochemistry (Kloss et al., 1991), and an APP-splitting cathepsin B-like activity was found in clathrin-coated vesicles derived from rat brain (Marks et al., 1994). In addition to lysosomes, cathepsin D is present in coated vesicles (Sapirstein et al., 1993), endosomes (Williams and Smith, 1993), trans-Golgi vesicles (Lammers and Jamieson, 1988), and transport vesicles (Krieger and Hook, 1992). Normal and/or aberrant processing of APP in acidic compartments outside lysosomes may involve nonlysosomal cathepsins, such as cathepsin E. This putatively ER-located aspartic proteinase (Finley and Kornfeld, 1994) has recently been shown to liberate amyloidogenic fragments from APP (Ladror et al., 1994) and to be abundantly present in neurons and neuritic plaques of AD brains, but not normal brains (Bernstein and Wiederanders, 1994). Regardless of the route of APP processing, β-peptide or β-peptide-bearing fragments must leave the cell to form extracellular amyloid fibrils. In the case of β-peptide generation at the ectomembrane, the cell releases β-peptide into the extracellular space, and the further fate of the peptide largely depends on the microenvironment surrounding the cell. Therefore, certain metals (Multhaup et al., 1994) and apolipoprotein E (*see below*) have been identified as aggregation-promoting factors. Intracellularly produced β-peptide possibly leaves the cell via exocytosis or is even released at nerve endings (Schubert et al., 1991). There is no doubt that degenerating neurons in AD liberate larger amounts of β-peptide and intermediate fragments than intact nerve cells owing to their membrane disintegrity. There are hints that microglial proteinases play roles in the extraneuronal degradation of those APP fragments. Microglia contain cysteine proteinases (Banati et al., 1993) and their inhibitors, the cystatins (Bernstein et al., 1992b; 1994a, c); Ii et al., 1993; Zucker-Franklin et al. 1987). Microglial cells have been identified as a common constituent of neuritic plaques (Wisniewski et al., 1991). In this respect, a contribution of microglia to the amyloid formation is well imaginable (Shigematsu et al., 1992).

NEUROFIBRILLARY DEGENERATION AND CATHEPSINS

Another defining feature of AD is intraneuronal neurofibrillary tangles of paired helical filaments (PHF) containing an admixture of 2.1-nm-thick

τ filaments (Ruben et al., 1991; Iqbal et al., 1994). Neurofibrillary changes may lead to functional disturbances and eventually to the death of the nerve cells. Dying neurons leave behind tangled masses of extracellularly located abnormal fibrils. These "ghost tangles" differ from intraneuronal tangles in their fibril composition (Iqbal et al., 1994). In AD brains, there exist two populations of PHF (PHF I and II) differing mainly in their solubility in sodium dodecyl sulfate and putatively representing early and late stages of the development of the neurofibrillary tangles (Iqbal et al., 1984; Hussey et al., 1987). τ-Protein, the major protein subunit of PHF, represents a family of neuronal polypeptides encoded on a single gene (Lee et al., 1993). The most characteristic feature of τ-protein in AD is its abnormally high degree of phosphorylation (Grundke-Iqbal et al., 1986). The normal function of τ is apparently to maintain the microtubule structure (Drubin and Kirschner, 1986). Abnormal phosphorylation is believed to impair this function. Moreover, strongly reduced levels of τ as observed in AD also contribute to microtubule dissembly (Iqbal et al., 1994). Since assembly of tubulin into microtubules is a obligatory prerequisite of normal axonal transport, chemically modified and reduced τ may be responsible for impairment of axonal flow and neurodegeneration in AD.

Recent immunohistochemical findings of Ii and coworkers (1993) have shown a close association of cathepsin B with intracellular neurofibrillary tangles in AD brains. The pathophysiological importance of this finding is not yet well understood, however. There is some evidence for an influence of proteinase inhibitors on the metabolism of τ (Ivy et al., 1989). Lysosomal proteinases might affect other constituents of the neuroskeleton, thereby possibly contributing to the pathologic feature of AD. Human brain cathepsin D is known to be capable of splitting human neurofilament proteins 70, 160, and 200 kDa (Nixon and Marotta, 1984; Banay-Schwartz et al., 1987), as well as the main microtubule-associated proteins, MAP I and MAP II and tubulin (Matus and Green, 1987; Johnson et al., 1991). Cerebral cathepsin D activity has repeatedly been shown to increase with age (Banay-Schwartz et al., 1987, 1992; Matus and Green, 1987) and to change its substrate spectrum depending on age and the pH (Matus and Green, 1987). Both factors are of importance in connection with AD (especially with regard to the possible leakage of proteolytic enzymes from lysosomes of aged animals; Nakamura et al., 1989). Further, artificial inhibitors of cysteine proteinases (leupeptin and E 64) have been reported to block anterograde-retrograde conversion of axonally transported vesicles in rat brain neurons (Sahenk and Lasek, 1988) and to depress reversed protein transport in lesioned frog nerves (Smith and Snyder, 1991). This may lead to an accumulation of neurofilaments in nerve terminals and of the age pigment lipofuscin (Roots, 1983; Ivy et al., 1984, 1989), and finally, to the degeneration of neuronal processes (Takauchi and Mioshi, 1991). However, these degenerated neurites do not contain PHFs (Takauchi and Mioshi, 1991), thus clearly differing from the typical feature of neurofibrillary pathology in AD. In contrast to these findings,

(1989) reported an accumulation of phosphorylated τ in rat brain after the application of leupeptin and E 64. Importantly, Ivy was able to show that these effects are owing to an inhibition of lysosomal cysteine proteinases rather than of serine proteinases or calcium-activated neutral proteinases. Shea (1994) observed an enhancement of phosphorylated neurofilament immunoreactivity in neuroblastoma cells after treatment of the cell culture with leupeptin.

Recent studies have clearly demonstrated that APP and APP fragments are integral parts of NFTs (Perry et al., 1993). These findings contribute to a more complex, unifying concept of AD pathology in that plaques and tangles are not separately developing features of the disease, but may be interrelated. One possible consequence is that the proposed role of cathepsins in the normal and/or aberrant processing of the amyloid precursor molecules may therefore have influence on the composition of NFTs.

APOLIPOPROTEIN E AND CATHEPSINS

In search for a genetic linkage for late-onset AD, evidence has been provided that apolipoprotein E (Apo E) allele $\epsilon 4$ is a susceptibility gene or at least a risk factor in both sporadic and familial late-onset AD (Schmechel et al., 1993; Strittmatter et al., 1993). Apo E (a 34-kDa glycoprotein) is part of the chylomicrons and very low-density and high-density lipoproteins, and serves for the transport and metabolism of cholesterol (Ye et al., 1993). In the CNS, Apo E seems to play roles in the growth, differentiation, and repair of myelin and axonal membranes. It is synthesized in astrocytes and microglial cells. Apo E has frequently been observed in amyloid deposits in plaques and vessels of AD brains and other amyloidoses. Apo E binds with high affinity to β-peptide and amyloid, thereby possibly forcing the growth of plaques (Hardy, 1994). The binding of the $\epsilon 4$ variant is much stronger than that of other Apo E forms, which might be the reason for its influence on AD (Förstl et al., 1994). Moreover, $\epsilon 4$ allele is known to increase circulating levels of cholesterol, which may induce AD-like β-amyloid immunoreactivity in the brain (Sparks et al., 1994). On the other hand, Apo E $\epsilon 2$ is even protective in that elderly Down Syndrome patients carrying this allele seem to live longer without having an increased AD neuropathology (Royston et al., 1994). The first report relating AD to both an increased expression of Apo E and cathepsin D came from Diedrich et al., (1991). Lysosomal proteinases have been shown to control the level of Apo E by the intracellular degradation of newly synthetized protein or after receptor-mediated reuptake (Van't Hofft et al., 1985; Ye et al., 1993; Jensen et al., 1994). By using specific blockers, Ye and coworkers have revealed that especially cysteine proteinases are involved in this regulatory mechanism (Ye et al., 1993). One may speculate that an imbalance between

cathepsins and cystatins as proposed in AD leads to an increased production of Apo E, which in the case of the genetically determined $\epsilon 4$ variant would further promote plaque formation. Evidence in favor of this assumption is still lacking, however.

CONCLUSIONS

Summing up, it can be said that cathepsins and cystatins may be involved in many neurodegenerative events occurring in AD brains. The neuronal endosomal-lysosomal system shows characteristic changes during early phases of AD development. These alterations are possibly triggered by pathologic changes occurring with neuronal membranes, which stimulate endocytosis and/or autophagy. The cathepsins D, B, and S, together with other lysosomal acid hydrolases, become upregulated, most probably as a consequence of the activation of the lysosomes.

If one supposes an important role for the endosomal-lysosomal pathway in the processing and/or degradation of the APP molecule on one hand, and a "cooperation" of aspartic and cysteine proteinases in the inactivation of (overexpressed?) APP on the other, one can speculate that an imbalance between the activities of these cathepsins may lead to an accmulation of potentially amyloidogenic fragments in nerve cells of AD brains. We believe that the "β-peptide-clearing" cysteine proteinases are inhibited in situations of AD by their naturally occurring blockers, the cystatins, whereas the "β-peptide-generating" aspartic proteinases are still highly active. This hypothesis needs experimental proof, however.

Potentially amyloidogenic fragments generated by lysosomal proteinases may be released into the extracellular space either by exocytosis or by lysis of the neuronal membrane when the neuron is dying. Interestingly, the acidic pH of endosomes-lysosomes drastically increases the formation of amyloid from amyloidogenic fragments. It is at present unkown if the cathepsins found in neuritic plaques may contribute to the formation and/or further growth of the plaque by proteolytic modification of the proteinacous material. We feel that in this context, the putative function of microglia-associated cathepsins and cystatins is currently underestimated and, therefore, warrants more attention.

Less is known about a possible involvement of cathepsins and cystatins in the generation of another characteristic hallmark of AD, the neurofibrillary tangles. Several cathepsins have been shown to accept neuroskeletal proteins as substrates, and cathepsin-immunoreactive material has been found in close association with tangles in AD neurons. Moreover, the administration of artificial blockers of cysteine proteinases produces changes of the neuronal cytoskeleton that are similar to those found in AD brains. Further investigations must be done to clarify whether or not lysosomal proteinases contribute to the tangle formation in AD brains.

Whether lysosomal proteinases and their inhibitors may serve as targets for drug intervention is yet difficult to say, since lysosomal proteinases play a fundamental role in the normal turnover of brain proteins and peptides, and any intervention should produce considerable side effects.

ACKNOWLEDGMENTS

The authors want to express their sincere thanks to A. M. Cataldo and R. A. Nixon (Belmont, CA) for their kind permission to reproduce and use microphotographs from one of their papers (Cataldo and Nixon, 1990). The technical help of I. Zipress, R. Luther, and H. Rummelfanger is gratefully acknowledged.

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