Virchows Archiv A Pathol Anat (1993) 423:185–194

Virchows Archiv A
Pathological Anatomy and Histopathology

Abnormal distribution of cathepsin proteinases and endogenous inhibitors (cystatins) in the hippocampus of patients with Alzheimer's disease, parkinsonism-dementia complex on Guam, and senile dementia and in the aged

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Received January 5, 1993 / Received after revision June 3, 1993 / Accepted June 8, 1993

Abstract. The immunolocalization of cathepsins B(CB), H and L and cystatins α(Cα) and β(Cβ) were examined in the hippocampus of cases of sporadic Alzheimer's disease (12 cases), parkinsonism-dementia complex on Guam (eight cases), senile dementia of Alzheimer type (two cases), aged subjects with marked senile change (one case) and controls (12 cases, including six normal subjects). CB was lower in most nerve cells in patients than in controls, but markedly increased at the sites of intracellular neurofibrillary tangles (NFTs) and degenerative neurites and/or dendrites in and outside senile plaques (SPs), indicating its close involvement in the metabolisms of various proteins in NFT and SPs. Abundant Cα and Cβ were demonstrated in SP amyloid, suggesting that they are amyloid constituents or co-exist with amyloid. The present study indicated that CB, Cα and Cβ are closely involved in abnormal protein metabolism in NFTs and SP amyloid and suggested that degeneration or denaturation of intracellular proteins, including substrates for proteases and lysosomes, from some acquired cause, results in absolute and/or relative overload for these proteolytic systems, including their inhibitors. This results in incomplete and/or abnormal proteolysis related to NFT and/or amyloid formation.

Key words: Cathepsins – Cystatins – Immunohistochemistry – Alzheimer's disease – Parkinsonism-dementia complex on Guam

Introduction

Neurofibrillary tangles (NFTs) and senile plaques (SPs) are characteristic histological hallmarks of the brain in Alzheimer's disease (AD; Alzheimer 1907; Tomlinson 1992), in some degenerative disorders such as Down's syndrome (Wisniewski et al. 1985), and in the aged (Scheibel 1978; Tomlinson and Corsellis 1984). The appearance of NFTs, usually not in association with SP, is a characteristic finding in the brain in amyotrophic lateral sclerosis (ALS)-parkinsonism-dementia complex on Guam (PDC; Hirano et al. 1961) and some disorders such as tuberous sclerosis (Hirano et al. 1968), meningioangiomatosis (Halper et al. 1978) and massive cerebral infarction (Kato et al. 1988). The presence of SP alone has been observed in disorders such as diffuse Lewy body disease (Okazaki et al. 1961; Dickson et al. 1989) and temporal lobe epilepsy (Munoz et al. 1990). These findings suggest that some factors are common to the formation of both NFTs and SPs, while others are not.

Recent biochemical, immunohistochemical and molecular genetic studies have clarified many characteristics of NFTs and SPs, including the protein constituents of NFTs, β/A4 amyloid protein (AP; Glenner and Wong 1984; Masters et al. 1985) and amyloid protein precursors (APPs; Kang et al. 1987; Kitaguchi et al. 1988; Tanzi et al. 1988) in SP amyloid. However, the pathogenesis of NFTs and SPs is still unknown.

We assume that abnormalities in the dynamic balance of protein metabolism in abnormal cells must be responsible for the formation of abnormal proteinaceous structures such as NFTs and SPs. Proteolytic systems including some proteinases and inhibitors are involved in this abnormal protein metabolism, but little is known about the involvement of these agents in the metabolism of the protein constituents in NFTs and SPs. There are no reports of immunohistochemical studies on cysteine proteinases or their endogenous inhibitors in the various disorders in which NFTs and/or SPs are formed.

In the present study, we examined the immunohistochemical localizations of cathepsins B(CH), H(CH) and L(CL), which are lysosomal cysteine proteinases, together with those of cystatins α(Cα) and β(Cβ), which are endogenous inhibitors of cysteine proteinases (Kirschke

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and Barrett 1987), in the hippocampus of patients with AD and related disorders. The possible roles of proteases and the inhibitors and the mechanisms in degradation and/or formations of NFTs and SP amyloid are discussed.

Materials and methods

Studies were made on autopsy specimens of the hippocampus from 12 patients with non-familial Alzheimer's disease (three men, nine women; age 75–95 years), eight with parkinsonism-dementia complex on Guam (five men, three women; age 35–68 years), two with senile dementia of Alzheimer type (SDAT; both women; age 82 and 86 years), and one 94-year-old woman with marked senile change. After confirmation of histological findings by review of all sections stained with H&E and silver impregnation (modified Bielshowsky). Ten serial sections of formalin-fixed, paraffin-embedded or unfixed frozen materials were prepared.

In addition, ten serial sections of formalin-fixed, paraffin-embedded or frozen specimens of histologically normal hippocampus from six subjects (four male, two female; age 6 months – 92 years) were examined as controls. Some of them were age matched. Furthermore, sections of two normal brains (cerebral cortex), one brain with fungal abscess, one brain with tuberculosis, three normal livers, two normal skins and one normal kidney were prepared to confirm the specificities of immunoreactions.

Most of the sections of hippocampus of both patients and controls included temporal cortex and medulla, choroid plexus cells, ependymal cells, macrophages and neutrophils, which also served as controls for immunoreactions.

CB, CH, CL and Cβ were purified from histologically normal fresh liver, and Cα was purified from histologically normal skin obtained at autopsy, according to previously reported methods (Kirschke et al. 1977; Towatari et al. 1979; Kominami and Katunuma 1982; Kominami et al. 1984; Bando et al. 1986).

Polyclonal antibodies (IgGs) were obtained by immunizing rabbits with these preparations. Anti-CB, -CH, -CL, -Cα and -Cβ antibodies were diluted to protein concentrations of 5, 10, 10, 10 and 10 μg/ml, respectively, for immunostaining. We confirmed previously that CB, CH and CL are immunochemically (Kominami and Katunuma 1982; Bando et al. 1986) and immunohistochemically (Ii et al. 1985, 1986) distinct, as are Cα and Cβ (Kominami et al. 1984).

Ten serial paraffin (4 μm thick) or frozen (8 μm thick) sections of each specimen were mounted on poly-L-lysine-coated slides. Sections 1, 2 and 3 were immunostained for CB, CH and CL, sections 4 and 5 for Cα and Cβ, while section 6 was stained with normal rabbit serum instead of a primary antibody as a control for each antibody. Sections 7–10 were reserved for use if the initial immunoreactions were unsatisfactory.

Paraffin sections were deparaffinized by warming them for 30 min at 60° C and rinsing them at room temperature three times in xylol, twice in 100% ethanol and then once in 90% ethanol and 80% ethanol for 5 min each time. Frozen sections were promptly fixed with 100% acetone for 5 min.

Immunostaining was performed by the avidin-biotin peroxidase complex (ABC) method as follows: deparaffinized or acetone-fixed frozen sections were rinsed for 5 min in PBS (0.1 M phosphate buffer, pH 7.4, containing 0.85% NaCl), treated with absolute methanol containing 0.1% hydrogen peroxide for 30 min to block endogenous peroxidase activity, and washed with PBS. They were then treated with normal goat serum in PBS for 30 min and reacted with rabbit anti-CB, -CH, -CL, Cα, or -Cβ or normal rabbit serum overnight at 4° C in a moist chamber. They were then washed with PBS and treated with biotinylated goat anti-rabbit IgG serum (Vectastain ABC kit, Vector) for 30 min, washed with PBS, and treated with avidin-biotin-horseradish peroxidase complex (Vectastain ABC kit, Vector). After washing with PBS, they were treated with 0.03% 3,3′diaminobenzidine tetrahydrochloride in 0.05 M TRIS-hydrochloric acid buffer, pH 7.6, for 10 min, washed with PBS, counterstained briefly with Mayer's haematoxylin solution or methyl green solution, dehydrated in 95% and 100% ethanol, cleared in carboxylol solution and xylol, and mounted with Entellan (Merck).

Results

The immunoreactivities of cathepsins and cystatins in frozen and formalin-fixed materials were similar, but somewhat stronger in the former. In specimens from both controls and patients, immunoreactivity for cathepsins was demonstrated as fine granules or vesicles of about 1–2 μm diameter, resembling lysosomes in structure. In contrast, immunoreactivity for cystatins was distributed diffusely in the cytoplasm.

In controls, nerve cells were mostly strongly reactive for CB (Fig. 1) and variously reactive for CL (Fig. 2) in their cell bodies, but not in their neuronal processes, including the dendrites and neurites (Fig. 1). CH was not detectable in nerve cells. Astro-, oligodendro- and microglia were essentially unreactive for CB, CH and CL, although very slight reactions were seen for CB in some oligodendroglia and for CL in some microglia.

Cα and Cβ were not detectable in nerve cells or glial cells. Macrophages present within and around blood vessels were strongly reactive for CB, CH, CL, Cα and Cβ and served as controls for immunoreactivity. CB, CH, CL, Cα and Cβ were present in various amounts in certain cells such as those in the choroid plexus and ependymal and endothelial cells, and these cells also served as positive controls of reactions for each antigen in the sections.

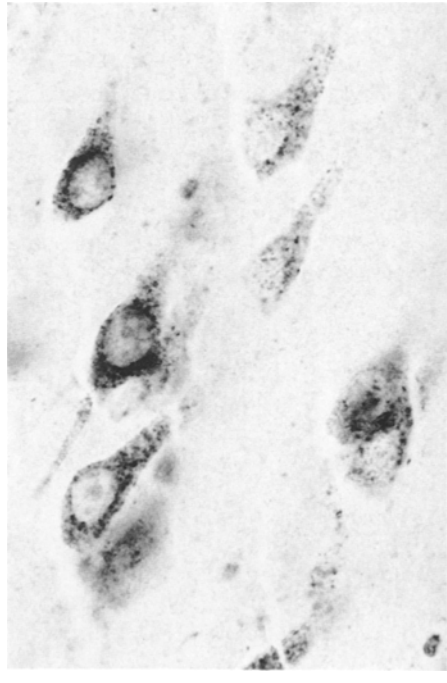

Fig. 1. Immunolocalization of cathepsin B (CB) in normal hippocampus (CA1 region) as control. CB is abundant in nerve cells and not present in glial cells. (× 700)

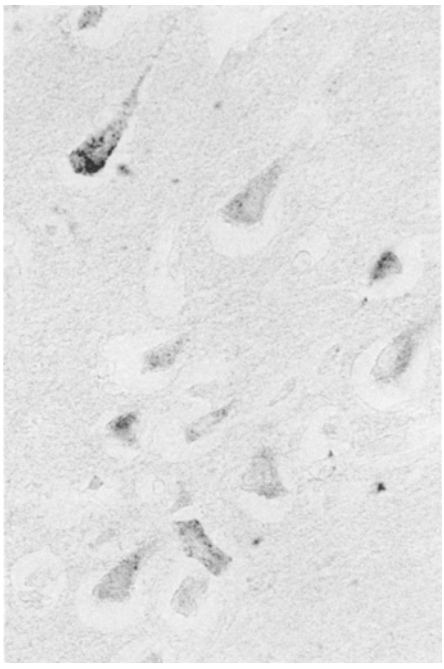

Fig. 2. Immunolocalization of cathepsin L (CL) in the Vth layer of the temporal lobe in the same section of hippocampus of normal brain. CL is abundant in some nerve cells but scarcely present, if at all, in others. (× 530)

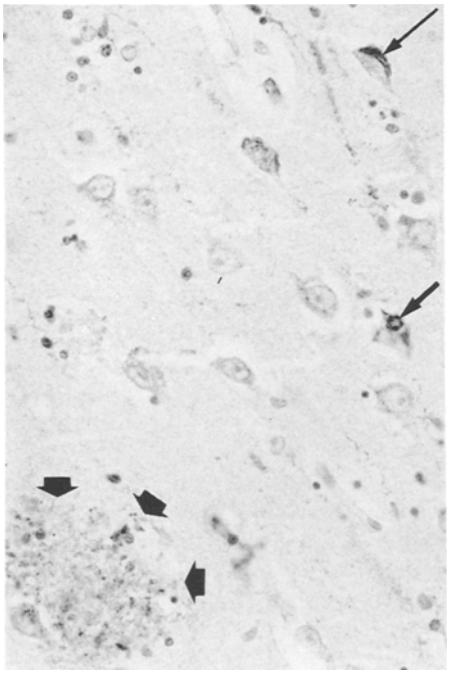

Fig. 3. Cathepsin B (CB) in the CA1 region of the hippocampus in Alzheimer's disease. CB is markedly increased in two intracellular sites, one sectioned longitudinally (*long arrow*) and the other transversely (*short arrow*) of neurofibrillary tangles (NFTs), in two nerve cells. It is predominant in the periphery of NFT in the cross section and discernible in the senile plaques (*thick arrows*), mostly in dystrophic neurites. CB is decreased in other nerve cells without NFT formation and is undetectable in the glial cells. (× 350)

These clearly different cell distributions of individual cathepsins and cystatins indicate their different roles in the cells and the different roles of various cells in the brain in physiological conditions. Liver and renal tubular epithelial cells were strongly reactive for CB, CH and CL, the reactivities showing different distributions, as reported previously for normal liver (Ii et al. 1985). These finding confirm the specificities of the antibodies.

Cα was abundant in epidermal cells of the skin and present at lower level in neutrophils in a brain abscess. Macrophages and epithelioid cells in a tuberculous lesion were also strongly or moderately reactive for CB, CH, CL, Cα and Cβ. These reactivities showed different distributions, again indicating the specificities of the antibodies. Sections stained with normal rabbit serum instead of the antibodies showed no immunoreaction products, confirming that the antibodies and the immunoreactions were specific.

In patients with AD, SDAT, PDC and elderly subjects with senile change, the numbers of nerve cells were decreased to a varying extent. Many nerve cells with and without NFTs appeared degenerative. The densities of astro- and microglia per unit field appeared to be increased variously in the hippocampus and temporal cortex relative to those in age-matched controls. Some glial cells in the corona of SPs appeared somewhat degenerative.

The immunoreactivities for cathepsins and inhibitors in nerve and glial cells varied to some extent from case to case but were basically similar.

NFTs were numerous in both AD and the case of PDC. Immunoreactivities for CB in most nerve cells with and without NFTs were impaired variously. Most intra-

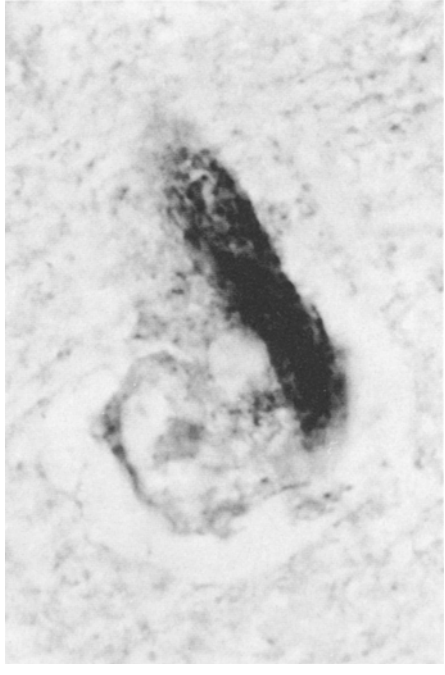

Fig. 4. High magnification of a nerve cell with a neurofibrillary tangle (NFT) in Alzheimer's disease. CB-positive granules are localized almost exclusively on or along the NFT. (× 1750)

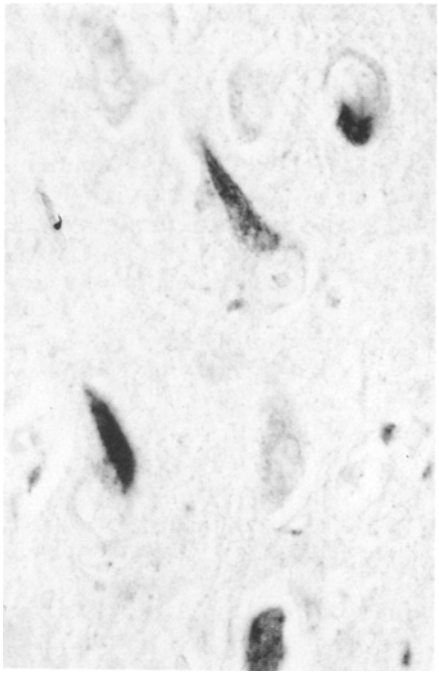

Fig. 5. Cathepsin B (CB) in nerve cells in the CA1 region of the hippocampus in parkinsonism-dementia complex on Guam. CB is abundant at the site of a neurofibrillary tangle (NFT) within some nerve cells but scarcely detectable in other nerve cells with no NFT. (×700)

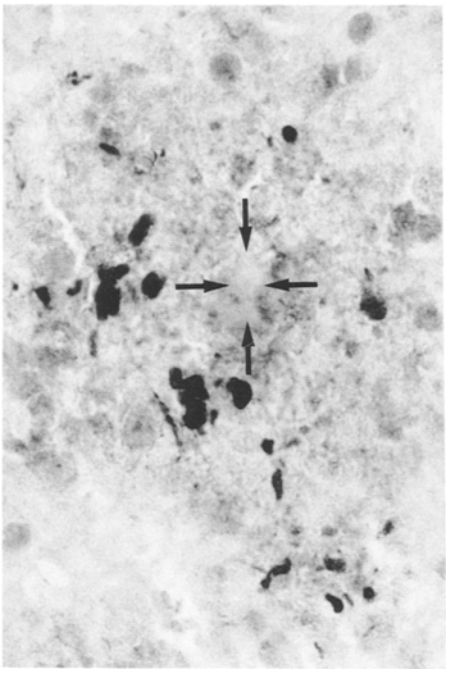

Fig. 6. Immunoreaction for cathepsin B (CB) in a typical (classical) senile plaque (SP) in parkinsonism-dementia complex on Guam. Abundant CB is present at the end of the degenerated neuronal processes in the periphery of the SP. An amyloid, which is observed as a vitreous homogeneous mass in the centre of the SP (*arrows*), is negative for CB. (Frozen section, ×700)

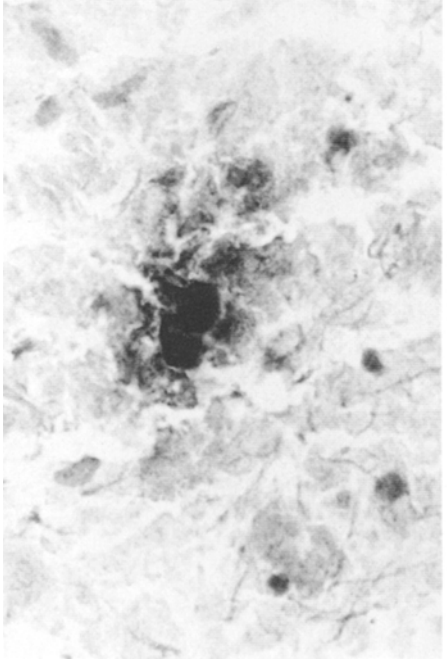

Fig. 7. Immunoreaction for cystatin α (Cα) in a senile plaque (SP) in the CA1 region in a patient with senile change. The amyloid in the centre of the SP is strongly positive for Cα. Cα is also present in the region adjacent to the amyloid mass. (Frozen section, ×700)

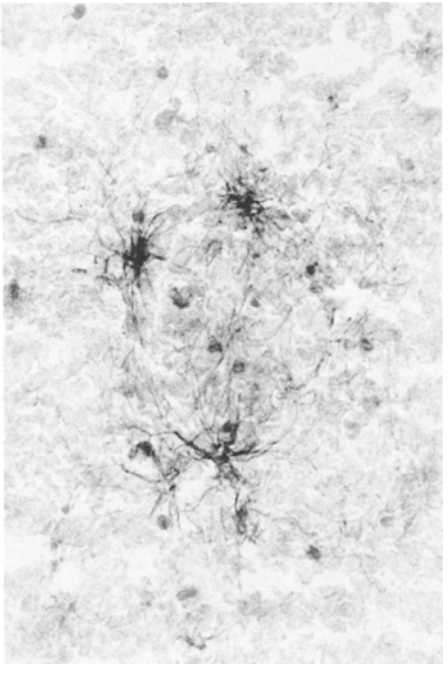

Fig. 8. Cystatin α (Cα) localization in and around a senile plaque (SP) in the CA1 region in senile change. Several reactive astroglia surrounding the SP are strongly reactive for Cα. Amyloid is not detectable in this SP. (Frozen section, ×350)

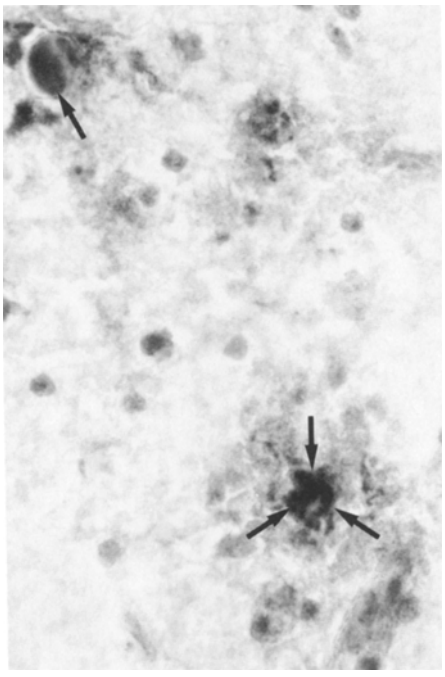

Fig. 9. Immunolocalization of cystatin β (Cβ) in two senile plaques (SPs) in the CA1 region in Alzheimer's disease. Abundant Cβ is present at the site of amyloid masses (top left and bottom right, *arrows*). The margin of an amyloid mass in the top left is smooth, while that of the other amyloid in the bottom right is irregular, indicating different stages of amyloid formation in SP. (Frozen section, × 700)

cellular NFT were very strongly reactive for CB in all diseases including AD (Figs. 3, 4) and PDC (Fig. 5). Some NFTs were weakly reactive for CL and Cα but none were reactive for CH or Cβ. Examination at higher magnification showed that the nerve cell cytoplasm was strongly reactive near the NFTs, but that the NFTs themselves were not reactive (Fig. 4). Extracellular NFTs were, however, unreactive for any cathepsin or cystatin.

SPs were numerous in AD, but not present in PDC except for all small number in one elderly patient with PDC. Degenerative neurites and/or dendrites in SPs were variously reactive for CB (Figs. 3, 6). Most astro- and/or microglia within and outside SPs were found to have increased levels of CB (Fig. 6) CH, CL, Cα (Figs. 7, 8) and Cβ (Fig. 9) in their cytoplasm. Amyloid masses, which were localized in the central portion of SPs and appeared as homogeneous vitreous globular masses, were strongly reactive for Cα (Fig. 7) and Cβ (Fig. 9), but no reaction for CB (Figs. 3, 6).

The corona of SPs were diffusely reactive for CB, Cα and Cβ (Figs. 3, 6, 7, 8).

The immunoreactions of these cathepsins and cystatins were qualitatively similar in all NFTs and SPs in different individuals regardless of the diseases, indicating that they are not disease-specific but lesion-specific.

Unlike the controls, in patients strong immunoreactivity for CB extended from the proximal to the distal portions of neurites in some degenerative nerve cells,

Table 1. Summary of immunoreactions in patients and controls

	Immunoreaction intensity				
	Cathepsin			Cystatin	
	B	H	L	α	β
In Patients					
Nerve cells					
Cell bodies	1–3	+−	0–3	1–2	+−
Neurites	(2–3)	+−	+−	+−	+−
Dendrites	(2–3)	0	+−	+−	+−
Glial cells					
Astro-	(2–3)	2–3	(2–3)	3	3
Oligo-	1–2	+−	0	+−	0
Micro-	3	3	2–3	+−	3
NFTs	4	+−	1–2	1–2	+−
SPs					
Amyloid	0	+−	+−	(3)	(3–4)
Neurites	3–4	+−	+−	+−	+−
Dendrites	+−	+−	+−	+−	+−
Astroglia	3	3	2	3	3–4
Oligodendroglia	0	+−	0	0	0
Microglia	2–3	2–3	2–3	3	3
In Controls					
Nerve cells					
Cell bodies	3	0	(2–3)	0	0
Neurites	+−	0	0	+−	+−
Dendrites	0	0	0	+−	+−
Glial cells					
Astroglia	+−	+−	+−	+−	+−
Oligoglia	+−	0	0	+−	0
Microglia	+−	0	3	+−	+−
NFTs[a]					
SPs[a]					

NFT, Neurofibrillary tangles; SP, senile plaques
0, absent; +−, ambiguous; 1, weak; 2, intermediate; 3, strong; 4, very strong
Parentheses = focal immunostaining
[a] not present in any sections

indicating its abnormal intracellular localization in these degenerative nerve cells. The immunoreactivities for CB and CL in the cell bodies of the nerve cells of most cases were less than those in normal hippocampus and temporal lobes used as controls.

Some or most astroglia had increased levels of CB, CH, CL, Cα and Cβ, some oligodendroglia had slightly increased levels of CB, and most microglia had markedly increased levels of CB, CH, CL, Cα and Cβ. The immunoreactivity in the patients is summarized in Table 1, by comparison with those in controls.

Discussion

CB, CH and CL are known to be lysosomal cysteine proteinases. Their biochemical characteristics have been

studied extensively (Kirschke et al. 1977; Towatari et al. 1979; Kirschke and Barrett 1987), but little is known about their roles in normal and pathological conditions in various organs, although we have reported their different localizations in normal liver (Ii et al. 1985), and their participation in autophagy in a vacuolar myopathy (Ii et al. 1986). Cα and Cβ are assumed to regulate the activities of cysteine proteinases in vivo, although there is as yet no direct evidence for this (Katunuma and Kominami 1985), and little is known about their roles in physiological and pathological conditions.

The different localizations of CB, CH, CL, Cα and Cβ observed in control brain in the present study indicate their different roles in normal brains. The localization of CB in nerve cells indicates its involvement in metabolism of proteins specific to nerve cells, such as various neuron peptides and neurofilamental and microtubular proteins. This immunohistochemical finding suggests that CB may be involved in processing APP as a secretase, because APP is localized in the perikarya of nerve cells (Schubert et al. 1991) and in lysosomes (Benowitz et al. 1989) and CB is biochemically assumed to be a secretase of APP (Tagawa et al. 1991). However, it is difficult to determine the true substrates of CB, because it has broad specificity as an endopeptidase, cleaving various sites of proteins, and a peptidase (Kirschke and Barrett 1987), and also acts as a hydrolase and a carboxydipeptidase (Katunuma and Kominami 1985). Details of the localization of cathepsins and cystatins in normal brain will be reported separately.

In this study on patients with AD and related disorders, the major abnormal findings were (1) a marked increase of CB at sites of NFTs and degenerative neurites in and outside SPs, (2) abundant Cα and Cβ in the amyloid core of SPs, (3) diffuse distribution of CB, Cα and Cβ in the corona of SPs, and (4) decrease of CB in most nerve cells.

Little is known about the intracellular proteolytic systems including lysosomal and non-lysosomal enzymes and inhibitors of proteases in nerve tissues. The marked increase of CB at sites of intracellular NFTs and dystrophic neurites in and outside SPs indicates that the levels of normal and/or abnormal protein substrates of CB are increased in these sites. There are recent reports of the presence of tau (Ihara et al. 1986; Dickson et al. 1989) and ubiquitin in NFTs and neurites of SPs (Perry et al. 1987; Dickson et al. 1989; Ito et al. 1991), tau and β/A4 AP in NFTs in AD (Hyman et al. 1989), low molecular weight proteins related or identical to those in amyloid filaments in paired helical filaments of NFTs (Selkoe et al. 1986), serum amyloid protein P in NFTs in AD (Coria et al. 1988), binding of APP to NFTs (Yamaguchi et al. 1990), β/44 AP and neurofilament determinants in the corona of SPs (Arai et al. 1990), β/A4 AP in the neurites of SPs (Yamaguchi et al. 1988), an epitope for NFTs in the N-terminus of the β/A4 AP molecule and an epitope of SP cores and vascular amyloid in the β/A4 AP molecule (Masters et al. 1985), and APPs at the sites of plaques in AD (Arai et al. 1990; Cras et al. 1991). There are also reports of production of a complex set of C-terminal derivatives of APPs, including the potentially amyloidgenic forms in the endosomal-lysosomal system (Golde et al. 1990), accumulation of APP in the lysosomal systems of dystrophic neurites in SP (Kawai et al. 1992) and degradation of ubiquitinated proteins in lysosomes (Doherty et al. 1989; Ueno and Kominami 1991). Therefore, the increase of CB observed in NFTs and dystrophic neurites suggests that CB is involved in the metabolism, production and/or degradation of these proteins, including tau, β/A4 AP, APPs and ubiquitinated proteins.

The high levels of Cα and Cβ observed in the amyloid core suggest that Cα and Cβ are also constituents of amyloid protein, that they co-exist with β/A4 AP, that they share some common epitope(s) with β/A4 AP, or that they adhere nonspecifically to amyloid. There are recent reports that not only β/A4 AP, but a hydrophobic low molecular weight protein in extracellular amyloid filaments in AD (Selkoe et al. 1986), domains for Kunitz-type serine protease inhibitor in some forms of APP (Tanzi et al. 1988; Kitaguchi et al. 1988) and a variant of cystatin C (a cysteine proteinase inhibitor like Cα and Cβ) in hereditary cerebral haemorrhage with amyloidosis, Iceland type (Ghiso et al. 1986) also, are amyloid proteins in the brain. α1-Anti-chymotrypsin (α/-ACT), a plasma serine protease inhibitor (Travis and Salvensen 1983), is thought to coexist with β/A4 AP in AD (Abraham et al. 1988) and disorders such as Down's syndrome, age-related cerebral amyloidosis, sporadic amyloid angiopathy, and hereditary cerebral haemorrhage with amyloidosis, Dutch type (HCHWA-D; van Duinen et al. 1987). Thus α1-ACT may participate in amyloid fibril production in AD (Miyakawa et al. 1992). It seems unlikely that Cα and Cβ share epitopes with β/A4 AP because of the differences between their amino acid sequences (Takio et al. 1983, 1984) and those of β/A4 AP (Glenner and Wong 1984). Non-specific adherence of Cα and Cβ to amyloid is also unlikely judging from their immunostainings. From these considerations, we think that Cα and Cβ are amyloid proteins or co-exist with amyloid.

From the diffuse staining of the corona of SP for CB, Cα and Cβ, it is unknown whether these substances are released or secreted from nerve cells or glial cells. There are reports that CB is secreted by macrophages (Morland and Pederson 1979; Sloane et al. 1986), neutrophils and some tumour cells (Sloane et al. 1986). Moreover, it is presumably released from cells together with the other lysosomal enzymes when the lysosomal system is stimulated (Kirschke and Barrett 1987). In the corona of SPs, the level of CB was decreased in the cell body but increased in the degenerative neurites and/or dendrites, astroglia and microglia. CB has an acidic pH optimum and functions in an acidic environment in the lysosomes and may act in the microenvironment just outside the cells where the pH conditions are under direct cellular control (Graf et al. 1981). Cα and Cβ are intracellular type proteins (Katunuma and Kominami 1985) lacking the signal peptides that are common to secretory type proteins (Takio et al. 1983, 1984). Thus Cα and Cβ in amyloid cores and diffusely distributed in the corona of SP may not have been secreted, but

have leaked pathologically from degenerative glial cells in which their levels are increased. Their leakage may cause neuronal degeneration, because similar degeneration of neuronal processes to that in senile plaques in AD was induced after injection of leupeptin, an inhibitor of various proteases, including CB, into rat brain (Takauchi and Miyoshi 1989; Nunomura and Miyagishi 1993), and they are very stable to extremes of pH (Kirschke and Barrett 1987) and so are presumably stable in the microenvironment just outside the cells. Thus, the present study indicates immunohistochemically that CB, Cα and Cβ are involved in amyloid formation and supports the idea that the corona of SPs may be the site of fragmentation of APPs (Arai et al. 1990).

The decrease of CB in most nerve cells in the patients compared with that in controls, indicating impairment or dysfunction of protein metabolism.

The four abnormalities described above were not disease-specific, being observed in most diseases and in aged subjects, indicating that similar abnormalities in protein metabolism are common to most diseases and during aging.

How can we understand these abnormal findings in relation to proteolysis in pathogenesis of NFTs and amyloid formation? Findings regarding the pathogenesis of SP and/or NFT formation in AD using transgenic mice (Kawabata et al. 1991; Quon et al. 1991; Wirak et al. 1991) are now controversial (Marx 1992). We think that qualitative and quantitative abnormalities of both the substrates and the proteolytic system must be important in the pathogenetic background of abnormal findings in the present study. These abnormalities may be both congenital and acquired.

Recent detection of amino acid substitutions of APP in some patients with familial AD (valine^{717} to isolencine; Goate et al. 1991; Naruse et al. 1991) and in HCHWA-D (glutamate^{619} to glutamine; van Duinen et al. 1987), and amyloid formation by transfection of C-terminal peptides of APP into cells (Maruyama et al. 1990) suggest that qualitative genetic (congenital) abnormality of APP as a substrate cause β/A4 amyloid production because of cleavage by proteases at abnormal sites.

Human trisomy-21 (the Down syndrome; Wisniewski 1985) and mouse trisomy-16 (Richards 1990) suggest that genetically determined quantitative excess of protease substrates are important for NFT and SP formations, because the amount of protein including APPs encoded by genes on the chromosomes in cases of trisomy is expected to be 150% of the normal amount.

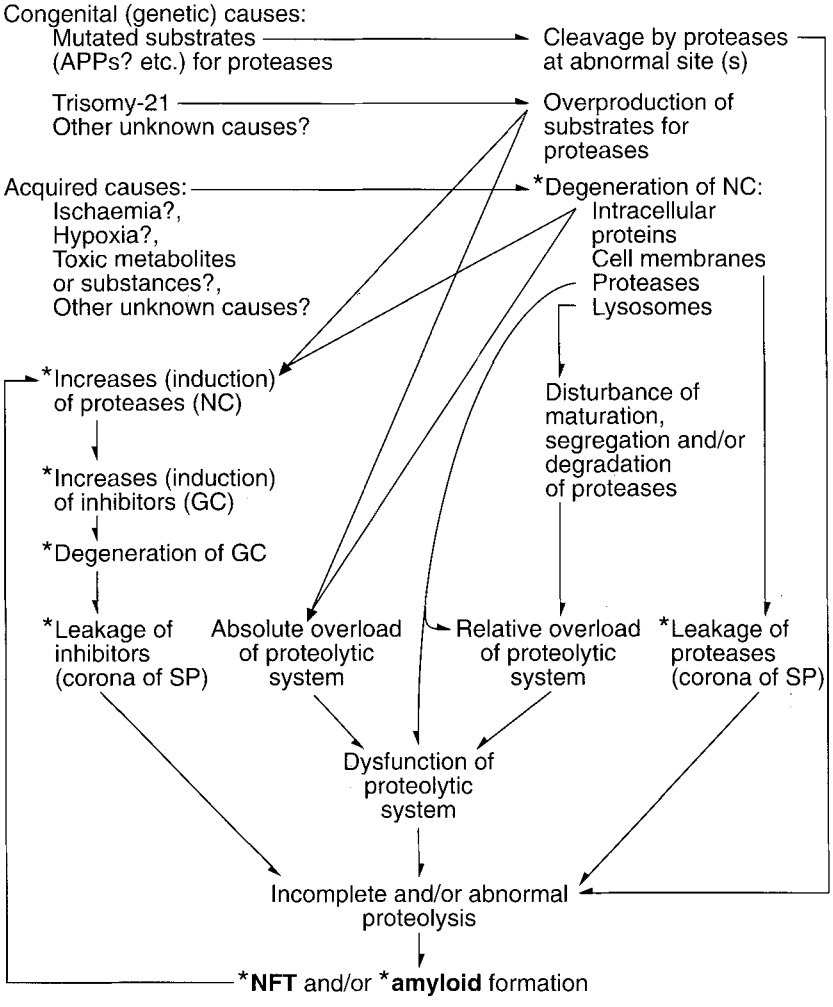

Fig. 10. Possible mechanism of proteolysis in neurofibrillary tangle (*NFT*) and amyloid formation. *APP*, amyloid precursor protein; *GC*, Glial cells; *NC*, nerve cells; *SP*, senile plaque; *, shown in this study

Qualitative genetic abnormalities of proteolytic systems are unknown. It is unlikely that susceptibilities to AD and abnormal processing of APPs are related genetically with abnormalities of the genes for CB, CH and CL, because the chromosomes with genes encoding CB, CH and CL (human gene mapping 10, 1989) are different from those with the AD gene in the early onset form (St. George-Hyslop et al. 1987), the AD gene in the late onset form (Pericak-Vance et al. 1991) and the APP gene (Korenberg et al. 1988), respectively. The sites of the genes of Cα and Cβ are unknown.

The facts that most cases of AD are sporadic and that similar histological alterations occur in the aged and that NFT formation occurs in cerebral infarction (Kato et al. 1988) indicate that some acquired disease processes, such as ischaemia, are also important. We believe that degeneration or denaturation of intracellular proteins and proteolytic systems including lysosomal and non-lysosomal protease systems are important. The former cause increase of proteases in response to increase in their substrates. The excess yield of substrate beyond the degradation capacity may cause incomplete or abnormal proteolysis as an absolute overload for the proteolytic system. The latter may cause incomplete or abnormal proteolysis as a relative overload for the proteolytic system following degeneration of proteases and lysosomes, especially in the nerve cells with impaired protease activity seen in the present study. We think that this incomplete or abnormal proteolysis, including abnormal processing and degradation of substrates for proteases, is responsible for NFT and amyloid formation. Our idea of the possible mechanisms of proteolysis in NFT and amyloid formation is summarized in the scheme in Fig. 10.

There are few reports of immunohistochemical studies of cathepsins in pathological brains: CB and CD (which is an aspartic proteinase) have been found in AD (Cataldo and Nixon 1990; Nakamura et al. 1991), and CB in aged human brain (Bernstein et al. 1990). There are no reports of immunohistochemical studies on CB in NFTs, or on Cα and Cβ in AD and related disorders, and no reports of comparison of the locations of CB, CH and CL and Cα and Cβ.

The present study suggests a possibility of treating AD and related disorders featuring NFT and/or SP formation with drugs that can control or reduce abnormal proteolysis.

Acknowledgements. The authors thank Dr. Dennis W. Dickson, Department of Neuropathology and Rose F. Kennedy Center for Research on Mental Retardation and Human Development and Neurology, Albert Einstein College of Medicine, Bronx, New York, for providing the frozen sections used in this study. This study was supported in part by grants from the Amyotrophic Lateral Sclerosis Association (USA) and Takeda Medical Research Foundation (Japan).

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