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Aggregation of β-Amyloid Peptide Is Promoted by Membrane Phospholipid Metabolites Elevated in Alzheimer's Disease Brain

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Abstract: Increased amounts of β-amyloid (Aβ) peptide deposits are found in Alzheimer's disease brain. These amyloid deposits have been implicated in the pathophysiology of this common dementing illness. Aβ peptides have been shown to be toxic to neurons in cell culture, and this toxicity is critically dependent on the aggregation of the peptide into cross-β-pleated sheet fibrils. Also, in vivo and postmortem NMR studies have shown changes in certain brain membrane phospholipid metabolites in normal aging and more extensive alterations in patients with Alzheimer's disease. The finding that membrane phospholipids affect the aggregation of Aβ suggests that the abnormalities in membrane metabolism found in Alzheimer's disease could affect the deposition of Aβ in vivo. Therefore, we examined the effect of membrane phospholipid metabolites that are altered in Alzheimer's disease brain on the aggregation of Aβ(1–40) using a light scattering method. Certain metabolites (glycerophosphocholine, glycerophosphoethanolamine, and α-glycerophosphate) augment the aggregation of Aβ. Other membrane phospholipid metabolites (phosphocholine, phosphoethanolamine, and inositol-1-phosphate) have no effect. We conclude that increased membrane phospholipid metabolite concentrations may play a role in the deposition of Aβ seen in normal aging and the even greater deposition of Aβ observed in Alzheimer's disease.
Key Words: Alzheimer's disease—β-Amyloid peptide—Aggregation—Phosphomonoester—Phosphodiester—Glycerophosphocholine.
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Neuropathologically, Alzheimer's disease (AD) is characterized by the presence of neuritic (senile) plaques, neurofibrillary tangles, and neuronal loss (Mann, 1985). Cerebral amyloid angiopathy is almost always present as well (Joachim et al., 1988; Wu et al., 1992). Neurofibrillary tangles are intracellular deposits of an abnormally hyperphosphorylated cytoskeletal protein called tau (Goedert, 1993). Neuritic plaques and cerebrovascular amyloid are extracellular deposits of the β-amyloid (Aβ) peptide (Prelli et al., 1988; Mori et al., 1992). Aβ is a 40–43-amino-acid fragment of a larger, ubiquitous class I transmembrane protein named the amyloid β-protein precursor (βPP). Although the exact mechanisms are not known, soluble Aβ is constitutively produced through normal processing of βPP (Haass et al., 1992; Seubert et al., 1992; Shoji et al., 1992). Another metabolic pathway converts βPP into a non-Aβ product through cleavage within the Aβ region by an enzyme that has been termed "α-secretase" (Esch et al., 1990; Sisodia, 1992).

Early in vitro studies demonstrated that Aβ peptide fragments have the inherent property of self-aggregation into fibrils (Castano et al., 1986; Kirschner et al., 1986, 1987; Gorevic et al., 1987). Hilbich et al. (1991) proposed a model in which Aβ forms a hairpin with two strands of antiparallel β-pleated sheets flanking a β-turn composed of residues 26–29. In structure–activity studies of a series of Aβ peptides, significant peptide aggregation was shown to be associated always with significant neurotoxicity (Pike et al., 1995). Therefore, understanding the aggregation of Aβ in vivo assumes critical importance and could lead to therapeutics aimed at preventing Aβ aggregation and toxicity.

Several groups have independently demonstrated

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Abbreviations used: Aβ, β-amyloid; AD, Alzheimer's disease; βPP, β-protein precursor; DMSO, dimethyl sulfoxide; α-GP, α-glycerophosphate; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; HFIP, hexafluoroisopropanol; I-1-P, inositol-1-phosphate; MRS, magnetic resonance spectroscopy; PBS, phosphate-buffered saline; PC, phosphocholine; PDE, phosphodiester; PE, phosphoethanolamine; PME, phosphomonoester.

changes in levels of membrane phospholipid metabolites in postmortem AD brain (Pettegrew et al., 1988*a*; Nitsch et al., 1992; Smith et al., 1993; Klunk et al., 1996). One of the most consistent findings is nearly twofold elevations in content of glycerophosphocholine (GPC), a phosphodiester (PDE) catabolic product of phosphatidylcholine metabolism (Nitsch et al., 1992; Klunk et al., 1996). In addition, postmortem magnetic resonance spectroscopy (MRS) studies have demonstrated changes in certain phosphomonoesters (PMEs), which are both precursors to membrane phospholipid synthesis and membrane breakdown products (Klunk et al., 1996).

Aβ aggregation has been shown to be affected by interactions with lipid vesicles that apparently stabilize the β-sheet conformation of Aβ through interaction with the head groups of phospholipids (Seelig et al., 1995). This finding has prompted interest in the possible effects of the PMEs and PDEs on the aggregation of Aβ because these molecules are phospholipid head groups cleaved free from the fatty acyl chains (Fig. 1). In this study, we examine the effect of selected PMEs and PDEs on Aβ aggregation as determined by a light scattering method (Jarrett and Lansbury, 1992; Jarrett et al., 1993). Understanding the role of altered endogenous metabolites on Aβ aggregation could lead to the development of agents that inhibit this aggregation. Such agents could have important therapeutic potential in the treatment of early AD.

MATERIALS AND METHODS

Materials

Aβ(1–40) was obtained from BACHEM Bioscience (King of Prussia, PA, U.S.A.). Two separate lots were used: one for the experiments shown in Figs. 2 and 4 and a second for the experiment shown in Fig. 3. Chemicals and solvents were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The naturally occurring L-isomers of GPC, glycerophosphoethanolamine (GPE), and α-glycerophosphate (α-GP) were used. The commercially available calcium salt of phosphocholine (PC) was converted to the sodium salt through ion exchange on a sodium-Chelex column (Bio-Rad Laboratories, Richmond, CA, U.S.A.).

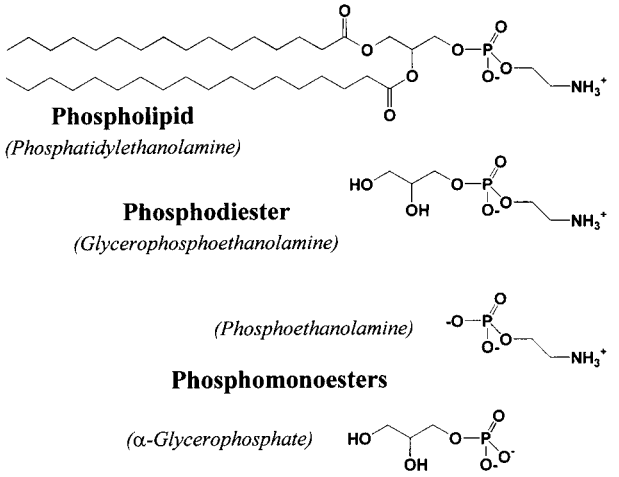

FIG. 1. Structural relationships among phospholipids, PDEs, and PMEs.

Aggregation studies

Phosphate-buffered saline (PBS) or Tris-buffered saline (100 m*M* NaCl and 10 m*M* sodium phosphate or Tris) was prepared fresh, adjusted to a pH of 7.4, and filtered through a nylon filter (pore size, 0.22 μm; Nylon-66; Supelco, State College, PA, U.S.A.). Aβ(1–40) was dissolved in hexafluoroisopropanol (HFIP) at a concentration of 1 mg/ml using sonication in an ice water bath for 5–10 min. Aliquots of the peptide in HFIP were dried under N_2 until a clear film formed. Additional drying results in the formation of a less soluble white solid (Jarrett and Lansbury, 1992; Jarrett et al., 1993). The dried peptide was dissolved in a volume of dimethyl sulfoxide (DMSO) equal to the original volume of HFIP and diluted with the PBS or Tris-buffered saline buffer to a ratio of 1:19 (DMSO:buffer). Eight hundred microliters of the peptide/buffer solution was added to the test compound in each cuvette immediately after preparation to initiate aggregation. Plastic tip pipettes were used for all sample measurements. The final peptide concentration was 11.3 μ*M*. Stock solutions (50 m*M*) of GPE, GPC, PC, phosphoethanolamine (PE), αGP, and inositol-1-phosphate (I-1-P) were prepared in distilled, deionized water and neutralized to pH 7.4 with 1 *M* NaOH or HCl as necessary before final volume adjustment. Test solutions (16.3 μl) were pipetted into 1.6-ml polystyrene semimicrocuvettes (Fisher Scientific, Pittsburgh, PA, U.S.A.) to give a final concentration of 1 m*M* after addition of the peptide/buffer solution. Appropriate adjustments in the GPC stock solutions were made for the concentration-dependency studies. The cuvettes were then sealed with Parafilm (Fisher Scientific) to prevent evaporation or seeding with airborne particulate material. Triplicate samples of each test solution were assayed. All samples to be compared were assayed simultaneously in one experiment. Cuvettes were inverted gently three times to suspend the fibrils, and absorbance was measured at 400 nm against a blank containing the buffer solution only (Jarrett and Lansbury, 1992; Jarrett et al., 1993). Samples were read at 4–12-h intervals over 16–18 days.

Electron micrographic studies

Samples were processed for transmission electron microscopy by adsorption of the samples obtained after aggregation onto Formvar-coated grids. Negative staining was performed by addition of freshly prepared and filtered 1% uranyl acetate to the grids.

Fluorescence studies

Samples of Aβ in PBS (either freshly prepared or after aggregation for 16 days) were centrifuged at 20,000 *g* for 10 min. Aliquots of the supernatant were incubated with a 10-fold molar excess of fluorescamine (Sigma). The fluorescamine-labeled samples were diluted 10-fold, and the fluorescence intensity was measured using an excitation wavelength of 385 nm and an emission wavelength of 490 nm.

RESULTS

Aβ aggregation was monitored by the light scattering method of Jarrett and Lansbury (1992). Special

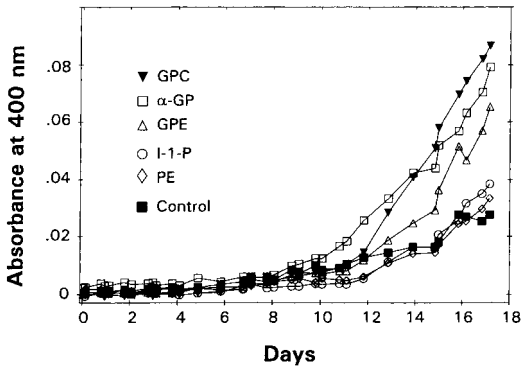

FIG. 2. Effect of selected PDEs and PMEs (1 m*M* concentration) on aggregation of 11.3 μM Aβ(1–40) in PBS. Error bars are omitted for clarity (see Fig. 3).

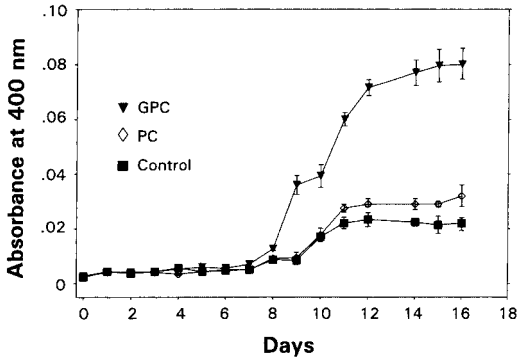

FIG. 3. Effect of PC and GPC (1 m*M* concentration) on aggregation of 11.3 μM Aβ(1–40) in Tris-buffered saline. Data are mean ± SEM (bars) values; error bars are representative of the variance observed in all experiments.

care was taken to ensure that each lot of peptide was converted to the α-helical form by dissolution in HFIP and DMSO before dilution in the aqueous aggregation buffer (Shen and Murphy, 1995). This is necessary because the initial β-sheet content of peptide preparations varies and affects the rate of aggregation (Shen et al., 1994; Howlett et al., 1995). All solutions were filtered to remove any particulate material >0.22 μm to minimize seeding the aggregation of Aβ. However, it is possible that particulates smaller than this could remain.

We observed a significant effect of several PMEs and PDEs on Aβ aggregation (Fig. 2). Both of the PDEs that have been shown to be elevated in content in AD brain (GPC and GPE) augment the aggregation of Aβ as determined by the light scattering method used. GPC appears to be more active than GPE. The PME α-GP also promotes aggregation in a manner similar to GPC. In contrast, the PMEs PE and I-1-P have no effect at 1 m*M* over this interval. Metabolites that promoted aggregation resulted in the appearance of large macroscopic aggregates in the cuvettes, compared with the control, which appeared cloudy with few macroscopic particles. The other abundant PME, PC, could not be adequately tested in the PBS system because it is available only as the calcium salt, and even after ion exchange for sodium, residual calcium forms precipitates in PBS, which result in light scattering in the absence of Aβ. Because of this, the effect of PC was determined in Tris-buffered saline. It can be seen in Fig. 3 that both the control and GPC samples aggregate in Tris-buffered saline in a manner very similar to that observed in PBS (Figs. 2 and 4). PC showed no significant effect on aggregation (Fig. 3). The aggregation-promoting effect of GPC was clearly concentration-dependent (Fig. 4). The variation in lag times between the aggregation studies shown in Figs. 2 and 4 may be due to differences in small (<0.22 μm) seeds that remain even after filtration of all solutions.

Electron micrographic studies of samples obtained from control and GPC are shown in Fig. 5. Both samples contained similar fibrils that had a width of ~10–11 nm. This is consistent with the previously reported dimensions of Aβ fibrils formed from synthetic Aβ (Howlett et al., 1995) as well as those found in plaques and cerebrovascular amyloid in AD (Roher et al., 1986) and Down's syndrome (Miyakawa et al., 1986). There was no evidence on electron micrographs of bacterial or fungal contamination.

Fluorescence studies indicated that a similar proportion of Aβ was converted from a soluble form to a form that could be pelleted at 20,000 *g* in the presence (56%) or absence (60%) of GPC.

DISCUSSION

There is considerable evidence that Aβ peptides are toxic to various cell types in culture (Yankner et al., 1990; Behl et al., 1992; Mattson et al., 1992; Giordano et al., 1994), suggesting that Aβ deposition may directly lead to neurodegeneration in AD. It is clear that aggregation of Aβ into macromolecular cross-β-pleated sheet fibrils is necessary for toxicity (Pike et al., 1991, 1993; Mattson et al., 1993; Lorenzo and

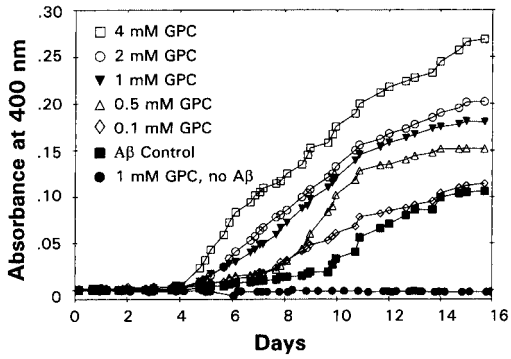

FIG. 4. Concentration dependence of the effect of 0.1–4.0 m*M* GPC on aggregation of 11.3 μM Aβ(1–40). Error bars are omitted for clarity (see Fig. 3).

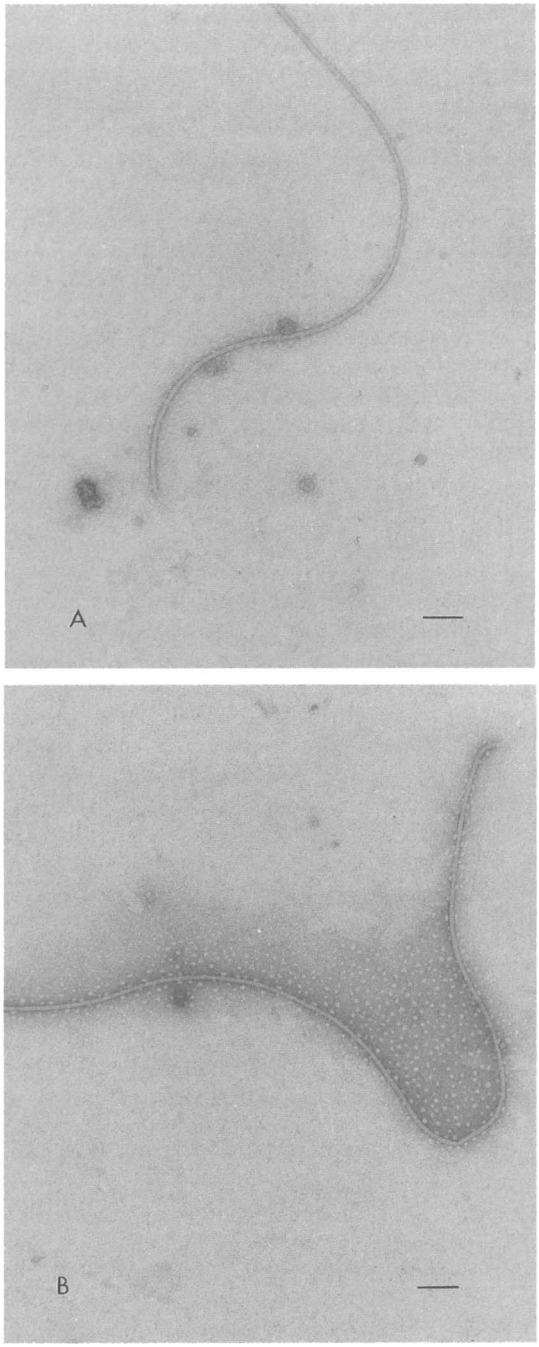

FIG. 5. Electron micrographs of Aβ aggregated in PBS buffer in the (**A**) presence or (**B**) absence of 1 m*M* GPC. Fibrils were negatively stained with 1% uranyl acetate. The dark area in B represents an accumulation of uranyl acetate around the fibril. Bars = 100 nm.

Yankner, 1994). A key question with regard to AD is how is the normal pathway of Aβ metabolism disrupted to result in the increased deposition of Aβ found in AD brains?

A complete understanding of the factors involved in the in vivo aggregation of Aβ may help answer this question. Detailed studies of the nucleation and extension of amyloid fibrils demonstrated that Aβ(1–40) forms peptide micelles at a critical micellar concentration <0.1 m*M*, fibrils nucleate within these micelles or on heterogeneous seeds, and growth of the fibrils occurs by irreversible binding of monomers to fibril ends (Lomakin et al., 1996). The initial nucleation-dependent formation of aggregates follows higher-order kinetics (Halverson et al., 1990; Jarrett and Lansbury, 1993; Jarrett et al., 1993) and may be affected by apolipoprotein E (Evans et al., 1995). Addition of radioiodinated Aβ to preexisting human brain amyloid deposits appears to show a first-order dependence on Aβ concentration and exhibits a pH optimum of 7 (Esler et al., 1996).

Sonicated lipid vesicles containing negatively charged phospholipids have been shown to shift the α-helix to β-sheet equilibrium of Aβ peptide solutions toward the β-sheet conformation (Seelig et al., 1995). This has been interpreted as resulting from a local increase in peptide concentration due to aggregation of the cationic peptides at the lipid/water interphase and ordering of the peptide molecules, both of which would facilitate β-sheet formation.

In this study, we investigate the effects of other membrane phospholipid metabolites on the aggregation of Aβ. We have found that specific membrane phospholipid metabolites augment Aβ aggregation. The two PDEs, GPE and GPC, are effective at concentrations found in AD brain (Pettegrew et al., 1988*a*; Klunk et al., 1996). For example, ^{31}P MRS studies show that GPC levels in normal aged brain are ~0.90 ± 0.05 μmol/g (or ~1.2 m*M* assuming 70% water content). AD brain has a GPC concentration of 1.61 ± 0.09 μmol/g (or 2.3 m*M*) (Klunk et al., 1996). We have observed levels as high as 4.7 μmol/g (~6.7 m*M*) in individual AD brain samples (W. E. Klunk et al., unpublished data). Similarly, GPE levels are 1.32 ± 0.05 μmol/g (1.9 m*M*) in AD brain compared with 0.93 ± 0.06 μmol/g (1.3 m*M*) in age-matched controls (Klunk et al., 1996). Individual AD brains have GPE levels as high as 2.5 μmol/g (3.6 m*M*) (W. E. Klunk et al., unpublished data). Similar aggregation-promoting effects were observed for the PME α-GP. In contrast, the other PMEs tested, PC, PE, and I-1-P, were without effect over the time frame of this experiment. It is possible that PC, PE, and I-1-P could have shown an effect if the aggregation were extended for longer times. Although there are no data in the literature on the concentration of α-GP in AD brain compared with age-matched controls, α-GP is known to increase with mild hypoxia in various tissues, including brain (Wardle and Riemersma, 1994; McClure et al., 1995*a*,*b*). PE content is decreased in AD brain (Ellison et al., 1987; Klunk et al., 1996). PC content is elevated to 0.60 ± 0.02 μmol/g in AD brain compared with 0.47 ± 0.04 μmol/g in control brain. I-1-P content is slightly elevated in AD brain, to a level

of 0.26 ± 0.02 μmol/g, compared with 0.21 ± 0.04 μmol/g in control brain (Klunk et al., 1996).

GPC, GPE, and α-GP all appeared to increase the final extent of fibril formation. This cannot be determined with certainty owing to the limitations of the light scattering method used. The scattering of light by a suspension of particles is not solely dependent on the number of particles present but also on their size. Therefore, the increased final light scattering seen with GPC, for example, could be due to the formation of aggregates with different physical characteristics, e.g., larger, from those formed in the absence of GPC. This is consistent with the presence of larger macroscopic aggregates in the samples aggregated in the presence of GPC than in control samples. Unfortunately, the electron micrographic studies are poorly suited to detect any quantitative physical difference between fibrils aggregated in the presence or absence of GPC. Studies using the technique of quasielastic light scattering spectroscopy previously reported by Lomakin et al. (1996) could better address this question. The fluorescence studies suggest a similar proportion of the total Aβ is converted to a form that could be pelleted at 20,000 *g* in the presence and absence of GPC. This suggests that the basis for the increased light scattering is more a difference in physical state than in the proportion of peptide that forms aggregates.

Based on the data discussed above, it is possible that these small, hydrophilic membrane phospholipid metabolites interact with Aβ in a manner that encourages the transition to the cross-β-pleated sheet structure. Because both the hydrophobic 29–40 and the more hydrophilic 1–28 regions have been implicated in the nucleation and stabilization of the cross-β-pleated sheet fibril (Halverson et al., 1990; Fraser et al., 1991; Hilbich et al., 1992; Wood et al., 1995), the PMEs and PDEs could interact with either or both fragments. Aggregation studies using these fragments in isolation could clarify the mechanism of action of the PMEs and PDEs in augmenting Aβ aggregation. In addition, experiments to determine the relative toxicity of Aβ fragments aggregated in the presence and absence of PMEs and PDEs could help delineate whether the effects observed in this study could result in Aβ deposits that are more neurotoxic.

Although the range of compounds studied is limited, several conclusions can be drawn about the structure–activity relationships involved in the aggregation-promoting effects of these phospholipid metabolites. All of the compounds that promoted Aβ aggregation (GPC, GPE, and α-GP) contain both glycerol and phosphate. The compounds that do not contain a glycerol moiety (PC, PE, and I-1-P) did not stimulate aggregation. The importance of the glycerophosphate portion of the molecule is evident when comparing the large difference in activity between GPC and PC or GPE and PE with the small difference in activity among GPC, GPE, and α-GP (see Fig. 1). Charge of the molecule does not seem to be a key feature. The phosphate pK_{a1} of PMEs and PDEs is ~2, and the pK_{a2} of PMEs ranges from 5.5 for PE to 6.3 for α-GP (Pettegrew et al., 1988*b*). The pK_a of the ethanolamine moiety is >9 (Pettegrew et al., 1988*b*). At the pH of the assay (7.4), the phosphate portion of the PDEs carries a −1 charge and that of the PMEs carries a charge of about −2. The ethanolamine and choline groups will both carry a +1 charge. The glycerol and inositol groups will be neutral. Therefore, under these conditions, GPC and GPE will have a net neutral charge, but α-GP will have a −2 charge. All of these compounds promote aggregation of Aβ. However, the net charge of the compounds that do not promote aggregation is in the same range. PC and PE will have a charge of approximately −1, and I-1-P will have a −2 charge.

These findings have important implications for the pathophysiology of AD. First, it is well known that aging is the single largest risk factor for AD, but there is no clear explanation why this is so. This is certainly true in the case of Aβ metabolism. Although it is clear that the deposition of Aβ is age-related, there is no evidence to suggest that there is a marked change in Aβ production with aging. Likewise, the genetic changes that are so closely associated with Aβ deposition are, of course, present throughout development. The reason for age-related Aβ deposition may relate to other age-related metabolic changes that impact on fibril formation. There are probably multiple such changes, but the progressive increase in levels of PDEs that has been demonstrated with aging (Panchalingam et al., 1990; Pettegrew et al., 1990; McClure et al., 1995*a*), coupled with the even greater increase observed in AD brain (Pettegrew et al., 1988*a*; Nitsch et al., 1992; Smith et al., 1993; Klunk et al., 1996), could play a role in increased Aβ deposition. It is possible that even subtle changes in the aggregation of this normally occurring peptide, integrated over years or decades, could result in the increased deposition observed in AD. If PMEs and PDEs do play a role in the age-related deposition of Aβ, then several new therapeutic approaches to the treatment of AD could be pursued. These would include strategies to decrease specific PME and PDE levels as well as the design of small molecules that would interfere with the tendency of PMEs and PDEs to augment Aβ fibrillogenesis.

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REFERENCES

Behl C., Davis J., Cole G. M., and Schubert D. (1992) Vitamin E protects nerve cells from amyloid beta protein toxicity. *Biochem. Biophys. Res. Commun.* **186,** 944–950.

Castano E. M., Ghiso J., Prelli F., Gorevic P. D., Migheli A., and

Frangione B. (1986) In vitro formation of amyloid fibrils from two synthetic peptides of different lengths homologous to Alzheimer's disease beta-protein. *Biochem. Biophys. Res. Commun.* **141,** 782–789.

Ellison D. W., Beal M. F., and Martin J. B. (1987) Phosphoethanolamine and ethanolamine are decreased in Alzheimer's disease and Huntington's disease. *Brain Res.* **417,** 389–392.

Esch F. S., Keim P. S., Beattie E. C., Blacher R. W., Culwell A. R., Oltersdorf T., McClure D., and Ward P. J. (1990) Cleavage of amyloid beta peptide during constitutive processing of its precursor. *Science* **248,** 1122–1124.

Esler W. P., Stimson E. R., Ghilardi J. R., Vinters H. V., Lee J. P., Mantyh P. W., and Maggio J. E. (1996) In vitro growth of Alzheimer's disease beta-amyloid plaques displays first-order kinetics. *Biochemistry* **35,** 749–757.

Evans K. C., Berger E. P., Cho C. G., Weisgraber K. H., and Lansbury P. T. Jr. (1995) Apolipoprotein E is a kinetic but not a thermodynamic inhibitor of amyloid formation: implications for the pathogenesis and treatment of Alzheimer disease. *Proc. Natl. Acad. Sci. USA* **92,** 763–767.

Fraser P. E., Nguyen J. T., Surewicz W. K., and Kirschner D. A. (1991) pH-dependent structural transitions of Alzheimer amyloid peptides. *Biophys. J.* **60,** 1190–1201.

Giordano T., Pan J. B., Monteggia L. M., Holzman T. F., Snyder S. W., Krafft G., Ghanbari H., and Kowall N. W. (1994) Similarities between beta amyloid peptides 1–40 and 40–1: effects on aggregation, toxicity in vitro, and injection in young and aged rats. *Exp. Neurol.* **125,** 175–182.

Goedert M. (1993) Tau protein and the neurofibrillary pathology of Alzheimer's disease. *Trends Neurosci.* **16,** 460–465.

Gorevic P. D., Castano E. M., Sarma R., and Frangione B. (1987) Ten to fourteen residue peptides of Alzheimer's disease protein are sufficient for amyloid fibril formation and its characteristic x-ray diffraction pattern. *Biochem. Biophys. Res. Commun.* **147,** 854–862.

Haass C., Schlossmacher M. G., Hung A. Y., Vigo-Pelfrey C., Mellon A., Ostaszewski B. L., Lieberburg I., Koo E. H., Schenk D., Teplow D. B., and Selkoe D. J. (1992) Amyloid beta-peptide is produced by cultured cells during normal metabolism. *Nature* **359,** 322–325.

Halverson K., Fraser P. E., Kirschner D. A., and Lansbury P. T. Jr. (1990) Molecular determinants of amyloid deposition in Alzheimer's disease: conformational studies of synthetic beta-protein fragments. *Biochemistry* **29,** 2639–2644.

Hilbich C., Kisters-Woike B., Reed J., Masters C., and Beyreuther K. (1991) Aggregation and secondary structure of synthetic amyloid βA4 peptides of Alzheimer's disease. *J. Mol. Biol.* **218,** 149–163.

Hilbich C., Kisters-Woike B., Reed J., Masters C. L., and Beyreuther K. (1992) Substitutions of hydrophobic amino acids reduce the amyloidogenicity of Alzheimer's disease beta A4 peptides. *J. Mol. Biol.* **228,** 460–473.

Howlett D. R., Jennings K. H., Lee D. C., Clark M. S., Brown F., Wetzel R., Wood S. J., Camilleri P., and Roberts G. W. (1995) Aggregation state and neurotoxic properties of Alzheimer beta-amyloid peptide. *Neurodegeneration* **4,** 23–32.

Jarrett J. T. and Lansbury P. T. Jr. (1992) Amyloid fibril formation requires a chemically discriminating nucleation event: studies of an amyloidogenic sequence from the bacterial protein OsmB. *Biochemistry* **31,** 12345–12352.

Jarrett J. T. and Lansbury P. T. Jr. (1993) Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? *Cell* **73,** 1055–1058.

Jarrett J. T., Berger E. P., and Lansbury P. T. Jr. (1993) The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry* **32,** 4693–4697.

Joachim C. L., Morris J. H., and Selkoe D. J. (1988) Clinically diagnosed Alzheimer's disease: autopsy results in 150 cases. *Ann. Neurol.* **24,** 50–56.

Kirschner D. A., Abraham C., and Selkoe D. J. (1986) X-ray diffraction from intraneuronal paired helical filaments and extraneuronal amyloid fibers in Alzheimer disease indicates cross-beta conformation. *Proc. Natl. Acad. Sci. USA* **83,** 503–507.

Kirschner D. A., Inouye H., Duffy L. K., Sinclair A., Lind M., and Selkoe D. J. (1987) Synthetic peptide homologous to beta protein from Alzheimer disease forms amyloid-like fibrils in vitro. *Proc. Natl. Acad. Sci. USA* **84,** 6953–6957.

Klunk W. E., Panchalingam K., McClure R. J., and Pettegrew J. W. (1996) Quantitative ^{1}H and ^{31}P MRS of PCA extracts of postmortem Alzheimer's disease brain. *Neurobiol. Aging* **17,** 349–357.

Lomakin A., Chung D. S., Benedek G. B., Kirschner D. A., and Teplow D. B. (1996) On the nucleation and growth of amyloid beta-protein fibrils: detection of nuclei and quantitation of rate constants. *Proc. Natl. Acad. Sci. USA* **96,** 1125–1129.

Lorenzo A. and Yankner B. A. (1994) Beta-amyloid neurotoxicity requires fibril formation and is inhibited by Congo red. *Proc. Natl. Acad. Sci. USA* **91,** 12243–12247.

Mann D. M. A. (1985) The neuropathology of Alzheimer's disease: a review with pathogenetic, aetiological and therapeutic considerations. *Mech. Ageing Dev.* **31,** 213–255.

Mattson M. P., Cheng B., Davis D., Bryant K., Lieberburg I., and Rydel R. E. (1992) Beta-amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. *J. Neurosci.* **12,** 376–389.

Mattson M. P., Tomaselli K. J., and Rydel R. E. (1993) Calcium-destabilizing and neurodegenerative effects of aggregated beta-amyloid peptide are attenuated by basic FGF. *Brain Res.* **621,** 35–49.

McClure R. J., Kanfer J. N., Panchalingam K., Klunk W. E., and Pettegrew J. W. (1995*a*) Magnetic resonance spectroscopy and its application to aging and Alzheimer's disease, in *Neuroimaging Clinics of North America* (George A., ed), pp. 69–85. W. B. Saunders, Philadelphia.

McClure R. J., Panchalingam K., Klunk W. E., and Pettegrew J. W. (1995*b*) ^{31}P MRS *in vitro* study of graded hypoxia in Fischer 344 rats. *Soc. Neurosci. Abstr.* **21,** 2004.

Miyakawa T., Katsuragi S., Watanabe K., Shimoji A., and Ikeuchi Y. (1986) Ultrastructural studies of amyloid fibrils and senile plaques in human brain. *Acta Neuropathol. (Berl.)* **70,** 202–208.

Mori H., Takio K., Ogawara M., and Selkoe D. J. (1992) Mass spectrometry of purified amyloid beta protein in Alzheimer's disease. *J. Biol. Chem.* **267,** 17082–17086.

Nitsch R. M., Blusztajn J. K., Pittas A. G., Slack B. E., Growdon J. H., and Wurtman R. J. (1992) Evidence for a membrane defect in Alzheimer disease brain. *Proc. Natl. Acad. Sci. USA* **89,** 1671–1675.

Panchalingam K., Pettegrew J. W., Strychor S., and Tretta M. (1990) Effect of normal aging on membrane phospholipid metabolism by ^{31}P *in vivo* NMR spectroscopy. *Soc. Neurosci. Abstr.* **16,** 843.

Pettegrew J. W., Moossy J., Withers G., McKeag D., and Panchalingam K. (1988*a*) ^{31}P nuclear magnetic resonance study of the brain in Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* **47,** 235–248.

Pettegrew J. W., Withers G., Panchalingam K., and Post J. F. (1988*b*) Considerations for brain pH assessment by ^{31}P NMR. *Magn. Reson. Imaging* **6,** 135–142.

Pettegrew J. W., Panchalingam K., Withers G., McKeag D., and Strychor S. (1990) Changes in brain energy and phospholipid metabolism during development and aging in the Fischer 344 rat. *J. Neuropathol. Exp. Neurol.* **49,** 237–249.

Pike C. J., Walencewicz A. J., Glabe C. G., and Cotman C. W. (1991) In vitro aging of beta-amyloid protein causes peptide aggregation and neurotoxicity. *Brain Res.* **563,** 311–314.

Pike C. J., Burdick D., Walencewicz A. J., Glabe C. G., and Cotman C. W. (1993) Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state. *J. Neurosci.* **13,** 1676–1687.

Pike C. J., Walencewicz-Wasserman A. J., Kosmoski J., Cribbs D. H., Glabe C. G., and Cotman C. W. (1995) Structure–activity analyses of β-amyloid peptides: contributions of the β25–

35 region to aggregation and neurotoxicity. *J. Neurochem.* **64,** 253–265.

Prelli F., Castano E., Glenner G. G., and Frangione B. (1988) Differences between vascular and plaque core amyloid in Alzheimer's disease. *J. Neurochem.* **51,** 648–651.

Roher A., Wolfe D., Palutke M., and KuKuruga D. (1986) Purification, ultrastructure, and chemical analysis of Alzheimer disease amyloid plaque core protein. *Proc. Natl. Acad. Sci. USA* **83,** 2662–2666.

Seelig J., Lehrmann R., and Terzi E. (1995) Domain formation induced by lipid–ion and lipid–peptide interactions. *Mol. Membr. Biol.* **12,** 51–57.

Seubert P., Vigo-Pelfrey C., Esch F., Lee M., Dovey H., Davis D., Sinha S., Schlossmacher M., Whaley J., Swindlehurst C., McCormack R., Wolfert R., Selkoe D., Lieberburg I., and Schenk D. (1992) Isolation and quantification of soluble Alzheimer's beta-peptide from biological fluids. *Nature* **359,** 325–327.

Shen C. L., and Murphy R. M. (1995) Solvent effects on self-assembly of beta-amyloid peptide. *Biophys. J.* **95,** 640–651.

Shen C. L., Fitzgerald M. C., and Murphy R. M. (1994) Effect of acid predissolution on fibril size and fibril flexibility of synthetic beta-amyloid peptide. *Biophys. J.* **67,** 1238–1246.

Shoji M., Golde T. E., Ghiso J., Cheung T. T., Estus S., Shaffer L. M., Cai X. D., McKay D. M., Tintner R., Frangione B., and Younkin S. G. (1992) Production of the Alzheimer amyloid beta protein by normal proteolytic processing. *Science* **258,** 126–129.

Sisodia S. S. (1992) Beta-amyloid precursor protein cleavage by a membrane-bound protease. *Proc. Natl. Acad. Sci. USA* **89,** 6075–6079.

Smith C. D., Gallenstein L. G., Layton W. J., Kryscio R. J., and Markesbery W. R. (1993) ^{31}P magnetic resonance spectroscopy in Alzheimer's and Pick's disease. *Neurobiol. Aging* **14,** 85–92.

Wardle C. A. and Riemersma R. A. (1994) Hypoxia-stimulated glycerol production from the isolated, perfused rat heart is mediated by non-adrenergic mechanisms. *Basic Res. Cardiol.* **89,** 29–38.

Wood S. J., Wetzel R., Martin J. D., and Hurle M. R. (1995) Prolines and amyloidogenicity in fragments of the Alzheimer's peptide beta/A4. *Biochemistry* **34,** 724–730.

Wu E., Lipton R. B., and Dickson D. W. (1992) Amyloid angiopathy in diffuse Lewy body disease. *Neurology* **42,** 2131–2135.

Yankner B. A., Duffy L. K., and Kirschner D. A. (1990) Neurotrophic and neurotoxic effects of amyloid beta protein: reversal by tachykinin neuropeptides. *Science* **250,** 279–282.