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The β-amyloid protein precursor of Alzheimer disease has soluble derivatives found in human brain and cerebrospinal fluid

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ABSTRACT **In this study, we use antisera to synthetic β-amyloid protein precursor (βAPP) peptides to identify, in human brain and cerebrospinal fluid (CSF), soluble ≈125- and ≈105-kDa derivatives of the βAPP that lack the carboxyl terminus of the full-length, membrane-associated forms. We show that the soluble ≈125-kDa βAPP derivative contains the Kunitz protease inhibitor domain, whereas the ≈105-kDa form does not, and we confirm that these two proteins are soluble βAPP derivatives by purifying each from human CSF and directly sequencing its amino terminus.**

The principal proteinaceous component of the amyloid deposited in senile plaques and cerebral blood vessels in Alzheimer disease (AD) is a 39- to 42-residue polypeptide (the β-amyloid protein βAP) (1–3) derived from a much larger β-amyloid protein precursor (βAPP) (4–7). As shown in Fig. 1, the βAPP gene produces at least three mRNAs (8–10) through alternative splicing of two exons (8). One of these exons encodes a 19-amino acid domain; the other encodes a 56-amino acid domain that is highly homologous to the Kunitz family of serine protease inhibitors. In each full-length precursor, the 39- to 42-residue βAP occurs as an internal sequence (Fig. 1), which extends from the extracellular region into the putative membrane-spanning domain (5, 11). Thus, it appears that proteolytic cleavage of the βAPP at both the amino and carboxyl termini of the βAP is necessary to generate the βAP found in amyloid deposits.

In previous studies (12, 13), we identified a set of ≈110- to ≈135-kDa membrane-associated proteins that represent full-length forms of the βAPP. Proteolytic cleavage of these full-length forms at either the amino or carboxyl terminus of the βAP (Fig. 1) would be expected to generate large soluble derivatives that could be functionally significant. There is, in fact, evidence that some membrane-associated proteins with structures similar to the βAPP undergo limited proteolysis close to their membrane-spanning domain to generate soluble amino-terminal derivatives that are functionally important [e.g., prepro-epidermal growth factor (14, 15) and growth hormone receptor (16)]. Thus, a reasonable first step in evaluating both the process of amyloid deposition in AD and the normal function of the βAPP is to determine whether soluble derivatives are produced from the membrane-associated forms of this protein. In this study, we examine this question by using antisera to synthetic βAPP peptides to identify soluble βAPP derivatives, which are then isolated and sequenced. Our results show that soluble Kunitz protease inhibitor (KPI)-containing (≈125 kDa) and KPI-free (≈105 kDa) derivatives of the βAPP are readily detected in human brain and cerebrospinal fluid (CSF). Some of these findings have previously been reported in preliminary form (17).

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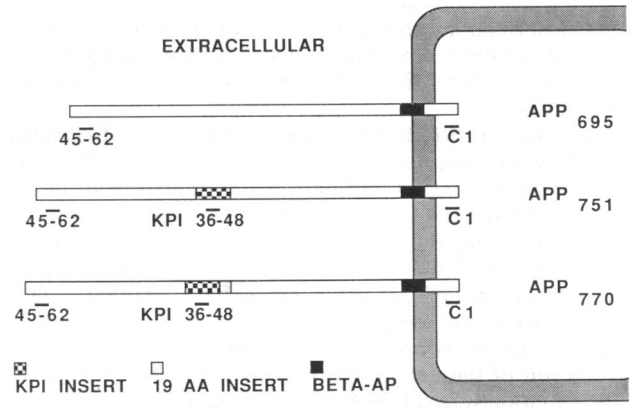

FIG. 1. Schematic structure of full-length 695-, 751-, and 770-residue forms of βAPP based on the model of βAPP^{695} proposed by Kang *et al.* (5). AA, amino acid.

MATERIALS AND METHODS

Antisera. To prepare antisera to KPI-containing forms of the βAPP (anti-KPI), keyhole limpet hemocyanin was linked to a synthetic peptide (Peninsula Laboratories) corresponding to amino acids 36–48 (KPI^{36-48}) in the KPI insert (9, 10). Production of antisera to the 20 carboxyl-terminal residues of the full-length βAPP (anti-C_1) has been described (12), as has (13) the production of antisera to amino acids 45–62 (βAPP^{45-62}) in the βAPP sequence (anti-N).

Brain Tissue. The tissue used in this report was dissected at autopsy, flash frozen, and stored at −80°C. Cerebral cortex in Figs. 3*B* and 4 and the nucleus basalis of Meynert in Fig. 4 *B* and *C* are from the brain of a control patient [age, 89 years; postmortem interval (PMI), 3 hr]; cerebral cortex used in Fig. 3*A* is from an AD patient (age, 85 years; PMI, 2 hr). For preparation of soluble and membrane-associated proteins, tissue was homogenized in 2 vol of TS buffer (50 mM Tris·HCl/150 mM NaCl/5 mM EDTA/2 mM phenylmethylsulfonyl fluoride/1 μg of leupeptin per ml/10 μg of aprotinin per ml/0.1 μg of pepstatin per ml/1 μg of 7-amino-1-chloro-3-tosylamido-2-heptanone per ml, pH 7.6), and the homogenate was centrifuged at 100,000 × *g* for 1 hr. The supernatant, which contains soluble proteins, was removed. In an effort to remove residual soluble forms from the pellet, it was homogenized in 2 vol of TS, centrifuged 1 hr at 100,000 × *g*, the supernatant was discarded, and the resulting pellet was rinsed with TS. Membrane-associated forms were then extracted by homogenizing the washed pellet in 2 vol of TS buffer containing 2% Triton X-100 and centrifuging for 1 hr at 100,000 × *g*.

Abbreviations: AD, Alzheimer disease; βAP, β-amyloid protein; βAPP, βAP precursor; KPI, Kunitz protease inhibitor; CSF, cerebrospinal fluid; PMI, postmortem interval.

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CSF. CSF in Fig. 3*D* is from two living AD patients (ages, 69 and 67 years) and two controls (ages, 66 and 76 years). CSF in Fig. 4*A* was collected (*i*) postmortem from a patient with AD (age, 71 years; PMI, 3.5 hr) and (*ii*) from a living control (age, 71 years). CSF samples were dialyzed against 1 mM phosphate buffer (pH 7.4), and the desalted protein was concentrated 20:1.

Transfected Cell Lines. The human 293 embryonic kidney cells stably transfected with a βAPP^{695} expression construct (see Fig. 3*C*) have been described (12). Transcription in these cells is driven by the human cytomegalovirus immediate early gene enhancer/promoter unit. The construct also contains the simian virus 40 t-splice and late polyadenylylation signal. Cells that express recombinant βAPP^{751} cDNA (see Fig. 2) were prepared similarly.

SDS/PAGE. Proteins were electrophoresed on 7% or 5–20% SDS/polyacrylamide gels (18) and then transferred to nitrocellulose (19). The nitrocellulose blots were analyzed by using 5% nonfat dry milk (Carnation) to block nonspecific binding of primary antibody and goat anti-rabbit IgG coupled to alkaline phosphatase (Promega) for immunodetection. To demonstrate specific immunolabeling, 50 μg of the appropriate unconjugated peptide was incubated at 4°C overnight with 1 μl of antiserum diluted, as indicated in the figure legends, with Tris-buffered saline containing 0.05% Tween 20 and 1 mg of bovine serum albumin per ml. The absorbed antiserum was then centrifuged at 16,000 × *g* for 5–10 min, and the supernatant was applied to blots.

Purification and Sequencing. To purify the ≈105-kDa protein, we used CSF obtained at autopsy from a patient with AD (age, 82 years; PMI, 3 hr) and stored at −80°C. The βAPP derivatives were separated from the bulk (75%) of the CSF protein by ammonium sulfate fractionation: (*i*) 59.3 g of $(NH_4)_2SO_4$ was added slowly to 151 ml of CSF (60% saturation) at 4°C; (*ii*) the mixture was allowed to stir for 30 min and was then centrifuged at 10,000 × *g* for 30 min; (*iii*) the resulting pellet was resuspended in 20 mM sodium phosphate (monobasic) buffer (pH 6.8) to a final volume of 2.5 ml and was desalted with a Pharmacia column PD-10. The resuspended desalted $(NH_4)_2SO_4$ pellet was then loaded onto a Mono Q column (Pharmacia) and the bound proteins were eluted with a linear gradient of 20 mM phosphate buffer, pH 6.8/0.05% Tween 20 and 20 mM phosphate buffer, pH 6.8/0.05% Tween 20/1 M NaCl. The soluble βAPP derivatives eluted from the column together in a well-defined peak at ≈60% (0.6 M NaCl) of the gradient. The fractions comprising this peak were pooled, concentrated, desalted, and subjected to preparative SDS/PAGE on an 8% gel. The ≈125- and ≈105-kDa derivatives were then separately excised and electroeluted. After concentration and desalting, the recovered ≈125- and ≈105-kDa proteins were loaded into separate lanes for SDS/PAGE on an 8% gel. The proteins were transferred to Immobilon for 1 hr at 0.5 A and visualized with Coomassie blue R-250 as described by Matsudaira (20). The more abundant ≈105-kDa protein was then sequenced by using the Applied Biosystems 477A Sequenator. To obtain sufficient protein to sequence the ≈125-kDa form, we pooled CSF from five patients: three with AD (ages, 89, 87, and 68 years; PMI, 3, 3, and 3.5 hr); one control (age, 67 years; PMI, 4 hr); and one live patient undergoing aneurysm repair (age, 44 years). Purification and sequencing were performed as described for the ≈105-kDa protein with the following modifications: ammonium sulfate fractionation was performed at 62.5% saturation, 5–15% gradient gels were used for SDS/PAGE, and transfer to Immobilon proceeded for 1.5 hr.

RESULTS

To identify KPI-containing forms of the βAPP, we raised a rabbit antiserum to the KPI domain (anti-KPI) and evaluated this antiserum by using whole cell homogenates from a human 293 embryonic kidney cell line that had been stably transfected with a full-length βAPP^{751} expression construct. Fig. 2 shows that three 115- to 140-kDa proteins labeled by anti-KPI were markedly increased in the transfected as compared to the control cells. These three proteins were also labeled by anti-N, an antiserum that detects a near amino-terminal domain in the membrane-associated forms of the βAPP, and anti-C_1, which recognizes the carboxyl terminus of the full-length forms of the βAPP. Absorption with appropriate peptides showed that the labeling of the three proteins was specific. These data establish that our antiserum to the KPI domain recognizes those forms of the βAPP that contain the KPI insert, and they show that these forms are also detected by our antisera to the amino and carboxyl termini of the full-length forms of the βAPP.

In an effort to identify soluble amino-terminal derivatives of the membrane-associated forms of the βAPP, we examined immunoblots of soluble proteins from human brain tissue and CSF labeled with anti-N, anti-C_1, and anti-KPI. In presenting these immunoblots, we discuss only those proteins that were specifically labeled—that is, proteins whose immunostaining was abolished by absorbing the antisera with appropriate peptides. We focus initially on the ≈125- and ≈105-kDa soluble proteins that we have identified as derivatives of the βAPP. We then discuss a number of smaller proteins that were specifically labeled by our antisera.

Fig. 3*A* shows that anti-N labeled soluble ≈125- and ≈105-kDa proteins in human brain. The labeling of these proteins was specific because it was abolished by absorption with the appropriate N-terminal peptide. The ≈125- and

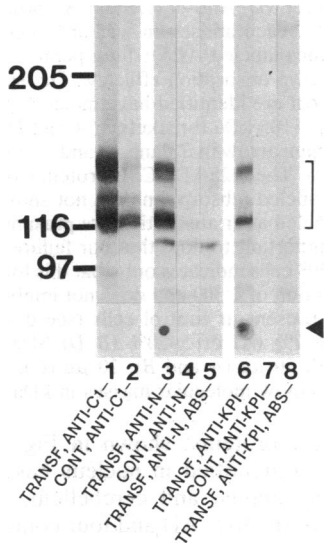

FIG. 2. Immunoblotted whole cell extracts of control human 293 embryonic kidney cells and 293 cells stably transfected with a full-length βAPP^{751} expression construct. Dilution was 1:200 for anti-N and anti-KPI and 1:300 for anti-C_1. In lanes labeled ABS, the antiserum was absorbed with the appropriate peptide; 1 μg peptide dots (arrowhead) were used to confirm absorption efficacy. The three large proteins (bracket) detected by anti-C_1, anti-N, and anti-KPI have calculated molecular masses of ≈140, ≈125, and ≈115 kDa. It is evident from the anti-C_1 immunolabeling (lanes 1 and 2) that the various membrane-associated forms of the βAPP are present at low levels in control cells and are markedly augmented in the transfected cells. Thus, the failure of anti-N (1:200) and anti-KPI (1:200) to detect the βAPP in control 293 cells indicates only that the level of the βAPP is sufficiently low that it is not detected by these antisera at the dilution used. Although not shown in this figure, anti-C_1 labeled a ≈14-kDa protein that was markedly increased in the transfected as compared to the control cells. Gel is 5–20% SDS/polyacrylamide; all lanes loaded with 75 μg of protein. Bars show molecular masses in kDa.

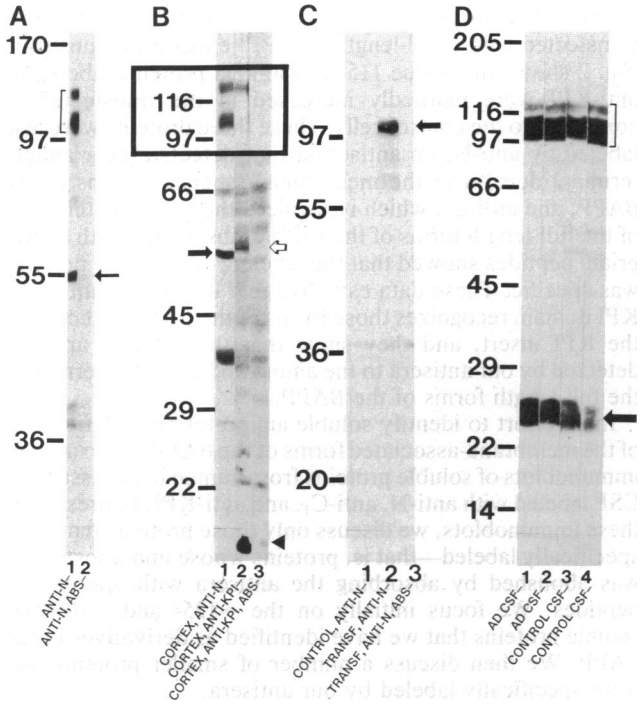

FIG. 3. (*A*) Anti-N (1:200) soluble proteins from human cerebral cortex. (*B*) Anti-KPI (1:100) vs. anti-N (1:100) soluble proteins from human cerebral cortex. (*C*) Anti-N (1:300) soluble proteins from control 293 cells vs. 293 cells transfected with a full-length βAPP^{695} expression construct. (*D*) Anti-N (1:100) CSF proteins. To evaluate labeling specificity, each antiserum was absorbed with the appropriate peptide (lanes labeled ABS); 1 μg peptide dots (arrowhead) were used to confirm absorption efficacy. The specifically labeled soluble proteins that are identified have molecular masses centered on ≈125 kDa and ≈105 kDa (brackets in *A* and *D*; box in *B*; arrow in *C*), ≈58 kDa (open arrow in *B*, lane 2), and ≈55 kDa (solid arrows in *A* and *B*, lane 1). The ≈25-kDa CSF protein (solid arrow in *D*) is also specifically labeled (absorption data not shown). Proteins that continue to be labeled after absorption are presumably unrelated to the βAPP. It is important to note that our failure to detect soluble βAPP in control 293 cells indicates only that this form is not detected by anti-N at a dilution of 1:300 and does not imply that this form of the βAPP is not present in control cells (see discussion in Fig. 2 legend). Gels are 7% (*A*) or 5–20% (*B–D*) SDS/polyacrylamide. Lanes loaded with 70 μg (*A* and *B*), 20 μg (*C*), or 130 μg (*D*) of protein. Numbers show molecular masses in kDa.

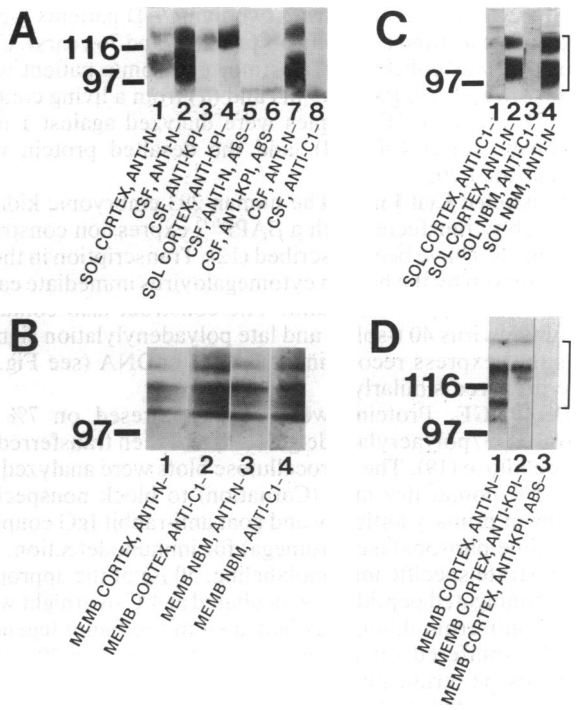

FIG. 4. (*A*) Soluble brain vs. CSF proteins. (*B*) Anti-N vs. anti-C_1 labeling of membrane-associated proteins from cerebral cortex and nucleus basalis of Meynert (NBM). (*C*) Anti-N vs. anti-C_1 labeling of soluble proteins from cerebral cortex and NBM. (*D*) Anti-N vs. anti-KPI labeling of membrane-associated proteins from cerebral cortex. Anti-N and anti-KPI were diluted 1:100; anti-C_1 was diluted 1:150. Gels are 5–20% (*A* and *D*) or 7% (*B* and *C*) SDS/polyacrylamide. Lanes loaded with 65 μg (*A*) or 70 μg (*B–D*) of protein. Numbers show molecular masses in kDa. The soluble proteins that are specifically labeled by anti-N but not by anti-C_1 in *A* and *C* have molecular masses centered on ≈125 and ≈105 kDa. In *B* and *D*, the two larger membrane-associated proteins detected by both anti-C_1 and anti-N, which are also labeled by anti-KPI, have calculated molecular masses of ≈133 and ≈120 kDa; the two smaller proteins detected by both anti-C_1 and anti-N but not by anti-KPI have calculated molecular masses of ≈115 and ≈110 kDa. The weights of these four membrane-associated proteins are essentially identical to those previously reported (12, 13).

≈105-kDa soluble proteins, shown in Fig. 3*A* in cerebral cortex, were also identified in nucleus basalis of Meynert (Fig. 4*C*), hippocampus, and cerebellum, and they were present in each of the four AD and four control patients that we examined. Fig. 3*B* shows that anti-KPI labeled a protein that comigrated with the soluble ≈125-kDa protein labeled by anti-N and that the labeling of this protein by anti-KPI was abolished after absorption with KPI^{36-48}. The fact that the ≈125-kDa soluble protein is specifically detected by two different antisera known to detect the βAPP, one directed against the KPI domain and the other against an amino-terminal domain, provides strong evidence that this protein is a derivative of the βAPP that contains the KPI domain.

To confirm that the soluble ≈105-kDa protein labeled by anti-N but not by anti-KPI is a KPI-free derivative of the βAPP, we examined the soluble proteins in a human 293 cell line designed to overexpress KPI-free βAPP through stable transfection with a βAPP^{695} expression construct (12, 13). As shown in Fig. 3*C*, this cell line produces markedly increased levels of a soluble ≈105-kDa form of the βAPP that is specifically detected by anti-N. This observation provides strong additional evidence that the soluble ≈105-kDa brain protein specifically detected by anti-N, but not by anti-KPI, is a KPI-free derivative of the βAPP.

Since our data showed that there are soluble derivatives of the βAPP in human brain tissue, we examined human CSF and found (Figs. 3*D* and 4*A*) that the soluble ≈125- and ≈105-kDa forms of the βAPP are present there as well. Fig. 3*D* shows that anti-N labeled ≈125- and ≈105-kDa proteins in samples of both AD and control CSF. As shown in Fig. 4*A*, anti-KPI labeled the ≈125-kDa CSF protein detected by anti-N, but it did not label the ≈105-kDa CSF protein detected by anti-N. The observed labeling of these CSF proteins by anti-N and anti-KPI was specific because it was removed by absorption with the appropriate peptides. The ≈125-kDa CSF protein labeled by anti-N and anti-KPI and the ≈105-kDa CSF protein labeled by anti-N but not by anti-KPI comigrated with their counterparts in human brain tissue.

As shown in Fig. 4*B*, both anti-C_1 and anti-N readily labeled four 110- to 135-kDa membrane-associated forms of the βAPP, as we had previously demonstrated (12, 13). Anti-C_1 did not, however, detect the soluble proteins that were labeled by anti-N either in CSF (Fig. 4*A*) or in brain tissue (Fig. 4*C*). Thus, our data indicate that there are soluble KPI-containing (≈125 kDA) and KPI-free (≈105 kDa) derivatives of the βAPP that lack the carboxyl terminus of the full-length membrane-associated forms. Moreover, we dem-

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~125 kDa form:  L E V P x x g n a g l l a

~105 kDa form:  L E V P T x G N A G L L A E P Q I A M F

Predicted βAPP: L E V P T D G N A G L L A E P Q I A M F
                18                                  37
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FIG. 5. Amino-terminal sequence (single-letter code) of the ≈105- and ≈125-kDa soluble proteins found in human CSF. Capital letters indicate that the amino acid signal was strong; lowercase letters indicate that the signal was weak although assignment was definite; x indicates that no amino acid could be identified. Note that the amino acid sequence predicted from βAPP cDNA (5) begins with a 17-residue signal sequence identified by Dyrks *et al.* (11). Thus the amino-terminal residue of the mature protein is located at position 18 of the predicted sequence.

onstrate that these soluble βAPP derivatives are readily detected both in brain tissue and in CSF.

To show definitively that the large soluble proteins identified by immunolabeling are derived from the βAPP, we sequenced the amino termini of the ≈105- and ≈125-kDa soluble proteins after having purified them from human CSF by ammonium sulfate fractionation followed by fast protein liquid chromatography using a Mono Q column (see *Materials and Methods*). As shown in Fig. 5, this sequencing confirmed that the ≈105- and ≈125-kDa proteins are forms of the βAPP. We sequenced 20 amino acids in the more abundant ≈105-kDa protein, 19 were detectable, and all 19 were identical to those predicted from the published βAPP cDNA sequence (5). In the ≈125-kDa protein, 13 amino acids were sequenced, 11 were detectable, and all 11 were identical to those predicted.

To further characterize the membrane-associated forms of the βAPP, we examined membrane-associated proteins from cerebral cortex with anti-KPI. Comparison of the membrane-associated proteins labeled by both anti-C_1 (Fig. 4*B*) and anti-N (Fig. 4 *B* and *D*) with those labeled by anti-KPI (Fig. 4*D*) showed that anti-KPI labeled two membrane-associated proteins that comigrated with the two largest membrane-associated forms of the βAPP. The labeling of these proteins by anti-KPI was specific because it was abolished (Fig. 4*D*) by absorption with the KPI^{36-48} peptide. The fact that anti-KPI specifically labels the two largest proteins detected by anti-C_1 and anti-N confirms that this antiserum detects the βAPP and shows that the two largest membrane-associated forms of the βAPP contain the KPI insert.

In addition to the soluble and membrane-associated proteins in the ≈105- to ≈135-kDa range, anti-N and anti-KPI specifically detect a number of proteins with molecular masses between ≈25 and ≈58 kDa. Anti-N specifically detects (*i*) an ≈55-kDa soluble protein in human brain (Fig. 3 *A* and *B*), (*ii*) an ≈55-kDa membrane-associated protein in human brain (13), and (*iii*) an ≈25-kDa protein in CSF (Fig. 3*D*; absorption with βAPP^{45-62} abolished this labeling; data not shown). Anti-KPI specifically detects an ≈58-kDa soluble protein (Fig. 3*B*) and an ≈58-kDa membrane-associated protein (data not shown) that are present in human brain extracts. The fact that these proteins are detected by antisera shown to recognize full-length forms of the βAPP suggests that the βAPP may be highly processed to produce a number of functionally important derivatives. At this point, however, these proteins do not meet the criteria required to establish that they are βAPP derivatives. Each of the ≈25- to ≈58-kDa proteins has been detected by only one antiserum, none appears to be augmented in transfected cells, and none has been sequenced. It is, therefore, possible that they are proteins, unrelated to the βAPP, which have cross-reacting epitopes detected by anti-N or anti-KPI.

SUMMARY AND DISCUSSION

In this report, we used three antisera—anti-N, anti-KPI, and anti-C_1—to demonstrate that there are large soluble KPI-containing and KPI-free forms of the βAPP that lack the carboxyl terminus of the full-length membrane-associated forms. Specifically, we identify (*i*) a soluble ≈125-kDa protein that is labeled by anti-N and anti-KPI but not by anti-C_1 and (*ii*) a soluble ≈105-kDa protein that is labeled by anti-N but not by anti-KPI or anti-C_1. We provide additional evidence that the ≈105-kDa protein is a KPI-free βAPP derivative by showing markedly increased levels of an ≈105-kDa soluble protein specifically detected by anti-N in a transfected cell line that overexpresses KPI-free forms of the βAPP. Both the KPI-containing (≈125 kDa) and KPI-free (≈105 kDa) βAPP derivatives are readily detected in human brain and CSF. We confirm that the large soluble proteins in human brain and CSF are derived from the βAPP by directly sequencing the amino terminus of both the ≈105- and ≈125-kDa forms. Finally, we examine the membrane-associated forms of the βAPP by using anti-KPI, and we show that the two largest membrane-associated forms of the βAPP contain the KPI domain.

The soluble KPI-containing and KPI-free βAPP derivatives identified in this study are ≈5–10 kDa smaller than the membrane-associated forms from which they are presumably derived; they are not detected by anti-C_1; and they do not require detergent for solubilization. Taken together, these observations indicate that these derivatives are generated by a proteolytic event that removes a small, 5- to 10-kDa fragment containing the carboxyl terminus and some or all of the hydrophobic membrane-spanning region from the full-length membrane-associated molecule. Since the predicted size of the fragments that extend from the amino and carboxyl termini of the βAP through the membrane-spanning domain to the carboxyl terminus of the βAPP are ≈11 kDa and ≈6.5 kDa, respectively, it is apparent that the postulated cleavage that generates soluble derivatives must occur in the vicinity of the βAP sequence (see Fig. 1). Thus, our data indicate that the βAPP, like other membrane-associated proteins with similar structure (14–16), undergoes limited proteolysis releasing soluble amino-terminal derivatives that, by analogy with these other proteins, may be functionally important.

The proteolytic event that generates soluble βAPP derivatives in human brain could occur on the amino-terminal side of the βAP, within the βAP sequence, or even on the carboxyl-terminal side of the βAP (Fig. 1). Cleavage on the carboxyl-terminal side of the βAP would be expected to shed a soluble, potentially amyloidogenic precursor into the extracellular space. This soluble βAPP derivative would have the βAP at or near its carboxyl terminus and would provide an extracellular source from which the amyloid deposited in AD, which is all extracellular, could readily be derived.¶

Cleavage on the amino-terminal side of the βAP, on the other hand, would leave the βAP attached to a small mem-

¶We have been unable to label the soluble derivatives of the βAPP by using antisera to the βAP. This observation cannot, however, be interpreted to mean that the soluble derivatives of the βAPP lack the βAP sequence because we, and many other groups, have been unable to label full-length membrane-associated forms of the βAPP by using antisera to the βAP. This failure may occur because synthetic βAP peptides, which spontaneously form amyloid fibrils (21, 22), have an immunogenicity that differs markedly from that of the βAP sequence in the native forms of the βAPP.

brane-associated protein from which amyloid might ultimately be generated. It is interesting, in this regard, that Dyrks *et al.* (11) have shown that this small protein tends to aggregate when it is transcribed and translated *in vitro* and that Selkoe *et al.* (12) have identified an ≈11-kDa membrane-associated protein in fresh cow and rat brain that could represent this fragment. It is apparent from the foregoing discussion that it is critically important to identify the specific site(s) at which the βAPP is normally cleaved to generate soluble forms. It should now be possible to do this by sequencing the carboxyl termini of soluble forms purified from the large volumes of CSF available at autopsy.

By carefully evaluating βAPP derivatives in CSF, it may be possible (*i*) to identify quantitative changes in βAPP derivatives or changes in the site(s) of βAPP cleavage that reflect significant pathogenic mechanisms operative in aging or AD and (*ii*) to monitor the progression of those changes in living patients. It has recently been shown that anti-C_1 (12), anti-N (13), and antisera to other βAPP peptides (23) label senile plaques. These findings suggest that full-length βAPP molecules may be processed into amyloid fibrils locally within plaques. There are typically >1400 plaques per mm^3 in AD cerebral cortex, far more than in control cortex, and these plaques have associated microglia and astroglia potentially capable of altering βAPP proteolysis. Thus, the process of plaque formation and amyloid deposition that occurs in AD may produce quantitative or qualitative changes in the βAPP derivatives present in CSF. Another factor that could alter βAPP derivatives in CSF is the selective increase in expression of the KPI-free form that we have observed in the neurons of certain populations in AD (24, 25). Finally, the loss of neurons that occurs in the AD brain might influence the level of βAPP derivatives in the CSF since these neurons are known to express the βAPP (26). It will, therefore, be important to analyze βAPP derivatives quantitatively in the CSF of a large number of AD and control patients. These quantitative studies and those characterizing the carboxyl terminus of the soluble derivatives could help clarify the mechanism responsible for amyloid deposition in AD, and they are necessary to determine the diagnostic utility of the βAPP derivatives that we have identified.

Noted Added in Proof. Since this report was submitted, Schubert *et al.* (27) have reported that PC12 cells secrete soluble βAPP derivatives of 115 and 140 kDa. Weidemann *et al.* (28) have also reported the presence of soluble forms of the βAPP in several cell lines and in human CSF. Moreover, Weidemann *et al.* have performed pulse–chase experiments that indicate that the soluble derivatives are generated from full-length membrane-associated βAPP. Finally, Takio *et al.* (29) have independently purified and sequenced three 110- to 130-kDa membrane-associated forms of the βAPP from newborn rat brain using a protocol similar to ours.

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