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Identification of a stable fragment of the Alzheimer amyloid precursor containing the β-protein in brain microvessels

(β-amyloid precursor protein/brain proteins/blood vessels/amyloidosis)

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ABSTRACT **Altered proteolysis of the β-amyloid precursor protein (βAPP) resulting in release of the ≈40-residue amyloid β-protein (AβP) may be a seminal pathogenetic event in Alzheimer disease. Using region-specific βAPP antibodies, we searched for stable proteolytic intermediates containing the intact AβP region in brain tissue. A 22-kDa βAPP fragment was selectively detected in microvessels purified from cerebral cortex and other brain regions. On immunoblots, the 22-kDa band is labeled by five distinct antisera to βAPP carboxyl-terminal peptides and by affinity-purified antibodies to the recombinant proteins $\beta APP_{444-592}$ and $\beta APP_{592-695}$, which flank the AβP region. The protein is virtually undetectable in whole-brain homogenates or microvessel-free fractions of brain. The protein is extractable from microvessels in Triton X-100 and other detergents, indicating its membrane association. In comparison with cortical microvessels, microvessels purified from white matter, cerebellum, and nonneural tissues contain lower amounts of the 22-kDa protein. The protein is found in microvessels of both normal and Alzheimer disease brains and occurs in low amounts in microvessels from fresh bovine brain. The size and specific immunoreactivity of the 22-kDa protein indicate that it is a stable fragment of βAPP containing the intact AβP. The occurrence of this potentially amyloidogenic intermediate in microvessels is consistent with a vascular or hematogenous origin for some AβP deposits in Alzheimer disease.**

Progressive cerebral deposition of the amyloid β-protein (AβP) in microvessels and parenchymal plaques is a principal feature of brain aging and Alzheimer disease (AD) (1–4). The 39- to 43-residue AβP consists of portions of two adjacent exons of the large, membrane-spanning β-amyloid precursor protein (βAPP) (5, 6). The AβP fragment must arise from proteolysis of βAPP; the responsible proteases are not yet known. We previously identified βAPP in brain, nonneural tissues, and cDNA-transfected cells as a complex group of 110- to 135-kDa membrane-associated proteins and described the occurrence of a stable ≈11-kDa proteolytic fragment that contains the carboxyl terminus (7). This fragment was subsequently purified by Esch *et al.* (8) from transfected cells and shown to result from a constitutive proteolytic cleavage at residue 612 of βAPP_{695} (residue 16 of AβP), enabling the secretion of the large amino-terminal portion of βAPP into the extracellular fluid. Some βAPP molecules must undergo alternative proteolytic processing during aging, and particularly in AD, to liberate intact AβP. Here, we report the identification of a stable ≈22-kDa fragment containing AβP and the carboxyl terminus of βAPP selectively in microvessels purified from brain.

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MATERIALS AND METHODS

Preparation and Analysis of Human Cerebral Microvessel Fractions. Fresh or frozen postmortem brain tissue was obtained from 16 patients with histologically confirmed AD (aged 64–89 yr), two Down syndrome patients (36 and 38 yr), and 18 non-AD subjects (fetal–78 yr). Frozen nonneural tissues (renal cortex, heart, spleen, and adrenal) and fresh or frozen animal brains (rat, guinea pig, rabbit, and cow) were also used to prepare microvessels. Microvessels were purified at 4°C by a modification of the method of Tsuji *et al.* (9). Cerebral gray matter (usually 5–10 g of wet weight) was minced into ≈2 mm^3 blocks, suspended in 5–10 vol of Tris-buffered saline (TBS) (50 mM Tris/150 mM sodium chloride/2 mM EDTA/0.1 mM diisopropyl fluorophosphate/leupeptin at 1 μg/ml/pepstatin at 0.1 μg/ml/7-amino-1-chloro-3-tosylamide-2-heptanone at 1 μg/ml, pH 7.6), and homogenized with a motor-driven, loosely fitting Teflon pestle (5–10 up-and-down strokes). The homogenate was sieved through 73-μm nylon mesh. The flow-through fraction was saved as the nonvascular brain fraction, and the mesh was washed in TBS buffer to resuspend the retained material. This suspension was placed on a column of 425- to 600-μm-diameter glass beads (Sigma) with a sheet of 73-μm nylon mesh at the bottom. The column was washed with 5–10 vol of TBS, and its contents were placed in a beaker of 50–100 ml of TBS. Microvessels were separated from the glass beads by agitation with a metal rod. The beads settled, and the microvessel suspension was decanted and centrifuged at 10,000 × *g* for 30 min. The resultant pellet was resuspended in 0.25 M sucrose, homogenized (five strokes), and layered on a discontinuous sucrose-density gradient (8 ml each of 1.0 M, 1.3 M, and 1.5 M sucrose in H_2O). After centrifugation at 58,000 × *g* in an SW 28 rotor for 120 min, the microvessel fraction was recovered as a pellet from the bottom of the tube and resuspended in 1–2 ml of TBS. Some microvessel fractions were prepared from fresh brain tissue by an alternate but comparable purification method described by Kalaria and Harik (10).

To separate large from small microvessels, the suspension retained on the 73-μm mesh was sieved through a 153-μm nylon mesh, and the latter was washed in TBS to resuspend the retained material. This suspension was centrifuged at 10,000 × *g* for 30 min at 4°C, and the resultant pellet was layered on a sucrose gradient as described above. For purification of small microvessels, the flow-through fraction off the 153-μm mesh was successively processed over the glass bead column and the sucrose gradient as described above.

Purified microvessel fractions were homogenized (10 strokes) in a Teflon homogenizer, mixed 3:1 with 4× SDS

Abbreviations: βAPP, β-amyloid precursor protein; FL-βAPP, full-length βAPP; AβP, amyloid β-protein; AD, Alzheimer disease.

sample buffer (11), sonicated for 10 sec, heated to 100°C for 5 min, and electrophoresed on 8-cm-long SDS/polyacrylamide minigels, usually using a 5–20% acrylamide gradient. Gels were blotted overnight at 150 mA or for 3 hr at 250 mA onto Immobilon (Millipore) (12). Duplicate protein determinations (13) on each microvessel fraction provided equal protein loadings for comparative immunoblots. Immunodetection used goat anti-rabbit or anti-mouse IgG secondary antibodies coupled to alkaline phosphatase (1:7500; Promega).

Antibodies. Immunoblots were probed with (*i*) affinity-purified rabbit antibodies to bacterially expressed recombinant proteins of either $\beta APP_{444-592}$ [αB5 (14); numbering according to βAPP_{695} (5)] or $\beta APP_{592-695}$ [αB6 (15)] or βAPP_{20-302} and (*ii*) antisera to carboxyl-terminal synthetic peptides comprising $\beta APP_{676-695}$ [αC1 (7), αC7, and αC8 (16)], $\beta APP_{681-695}$ [αC2 (17), from T. Ishii, Psychiatric Research Institute, Tokyo] and $\beta APP_{666-695}$ (αC4, from Y. Ihara, Tokyo University). A monoclonal antibody to recombinant $\beta APP_{444-592}$ (1G5) has been described (18). Two antibodies to AβP were used: a high-titer rabbit antiserum (1280) to synthetic $A\beta P_{1-40}$, produced as described (19) for our antiserum Y to $A\beta P_{1-38}$, and a monoclonal antibody (6C6) to synthetic $A\beta P_{1-28}$, from D. Schenk (Athena Neurosciences). All antibodies were routinely characterized on immunoblots of human kidney 293 cells transfected with either βAPP_{695} or βAPP_{751} cDNAs (7, 15). The specificity of all antibody reactions was established by peptide absorptions, usually 6–15 μg of peptide per μl of undiluted antiserum (7, 16).

RESULTS

Morphology and Yield of Cerebral Microvessel Fractions. Cerebral microvessel fractions purified as described were examined by phase-contrast microscopy. Abundant small and large microvessels ranged from 8 to 200 μm in diameter (Fig. 1), indicating that capillaries, arterioles, and venules were included. There was little contamination with light microscopically visible tissue debris. Fractions prepared from normal or AD cortex were morphologically indistinguishable, except for the presence of Congo red-positive microvessels to various extents in the AD fractions. The filtrate that passed through the 73-μm nylon mesh contained almost no vascular elements. Protein yield in the final fractions varied from 100 to 700 μg/g (wet weight) of starting cortex. By immunocytochemistry, the fractions were virtually unreactive for glial fibrillary acidic protein, a marker for astrocytes.

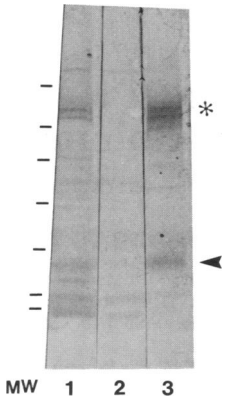

FIG. 2. Immunoblot of homogenates of human cerebral cortical microvessels (10 μg of protein per lane) treated with region-specific βAPP antibodies. A 5–20% acrylamide gradient minigel was blotted onto Immobilon. Lanes: 1, αC7 ($\beta APP_{676-695}$) (1:750); 2, αC7 after absorption with its corresponding peptide; and 3, affinity-purified αB5 ($\beta APP_{444-592}$) (1:1500). In addition to the characteristic labeling of the 110- to 135-kDa FL-βAPP proteins (asterisk), a 22-kDa protein (arrowhead) is specifically stained by αB5 and by αC7 before, but not after, peptide absorption. The 19-kDa band labeled by αC7 in lane 1 was only detected by this antibody, not by other antibodies to the βAPP carboxyl terminus; therefore, this band is unlikely to represent a βAPP fragment. M_r (MW) standards are indicated by bars (in descending order): 196, 106, 71, 44, 28, 18, and 15 ($\times 10^{-3}$).

A 22-kDa Protein Is Specifically Detected in Cerebral Microvessels by Numerous Antibodies to the βAPP Carboxyl Terminus. Homogenates of microvessels purified from control and AD cerebral cortex were probed on immunoblots with numerous region-specific βAPP antibodies. Anti-C7 specifically labeled several bands at ≈110–135 kDa that represent full-length (FL-) βAPP (7) (Fig. 2, asterisk). In addition, a band

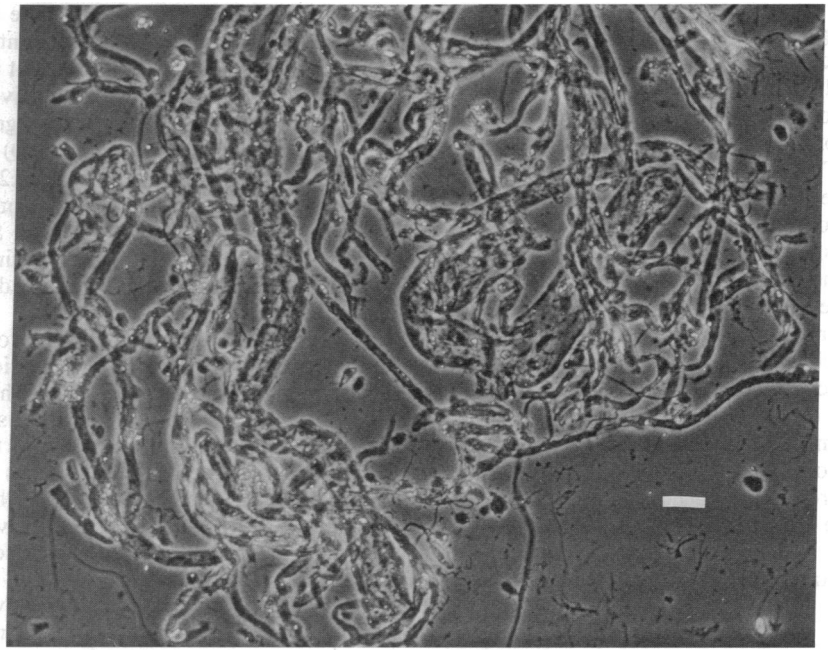

FIG. 1. Phase-contrast photomicrograph of isolated human cortical microvessels, purified as described. The vessels range in diameter from 8 to 200 μm. (Bar = 50 μm.)

migrating at 22 kDa was detected before, but not after, absorption of αC7 with its peptide immunogen (Fig. 2, lanes 1 and 2). Four other antisera to carboxyl-terminal synthetic peptides (αC1, αC2, αC4, and αC8) and an affinity-purified antibody to recombinant $\beta APP_{592-695}$ specifically labeled the 22-kDa band (see below). The 22-kDa fragment was also strongly reactive with affinity-purified $\alpha\beta APP_{444-592}$ (Fig. 2, lane 3) but did not react with affinity-purified $\alpha\beta APP_{20-302}$. Absorption of $\alpha\beta APP_{444-592}$ with the recombinant protein used as its immunogen (14) abolished the labeling of both the 22-kDa band and FL-βAPP. The band was reproducibly detected in microvessels isolated from either fresh or frozen postmortem human cortex using the procedure in *Materials and Methods* or the method of Kalaria and Harik (10).

Sequential extractions of the microvessel fraction revealed that both the ≈110- to 135-kDa FL-βAPP bands and the 22-kDa protein were virtually undetectable in the post-100,000 × *g* supernatant of a TBS extract but were extracted from the TBS-insoluble pellet by 1% Triton X-100 or 1% Brij 35 (Fig. 3*A*, lane 4). Small amounts of both FL-βAPP and the 22-kDa protein were retained in the Triton-insoluble pellet. The 22-kDa protein was not detectable in the microvessel-free brain fraction (i.e., the flow-through fraction off the 73-μm mesh; see *Materials and Methods*) (Fig. 3*A*, lane 3) or in the pia/arachnoid membrane stripped of visible microvessels (data not shown), consistent with its apparent localization to blood vessels. The 22-kDa protein was partially purified from the 1% Brij 35 detergent extract by Q Sepharose anion-exchange chromatography. Immunoblotting of the column fractions showed that the 22-kDa protein eluted at ≈0.4 M NaCl (Fig. 3*A*, lane 5), slightly before the peak elution of FL-βAPP. SDS/PAGE of this eluate in preparation for protein sequencing failed to reveal a Coomassie blue-reactive band at the 22-kDa position.

Q Sepharose fractions with the strongest immunoreactive 22-kDa protein were screened on immunoblots with numerous region-specific βAPP antibodies (Fig. 3*B*). All antisera to carboxyl-terminal synthetic peptides (αC1, αC2, αC4, αC7, and αC8), as well as affinity-purified antibodies to recombinant $\beta APP_{592-695}$, labeled the 22-kDa protein (Fig. 3*B*, lanes 2–10). Peptide absorptions selectively abolished the staining of both the 22-kDa band and FL-βAPP (Fig. 3*B*, lanes 4, 6, 8, and 10). The preimmune sera of αC1, αC2, αC7, and αC8 all failed to label either FL-βAPP or the 22-kDa protein. The 22-kDa fragment was again strongly labeled by affinity-purified $\alpha\beta APP_{444-592}$ (Fig. 3*B*, lane 1) but not by $\alpha\beta APP_{20-302}$. To ensure that the same protein, rather than two distinct comigrating proteins, was labeled by αC7 and $\alpha\beta APP_{444-592}$, microvessel extracts were bound to an αC7–protein A-agarose immunoaffinity column. The 22-kDa protein that eluted from this column was specifically labeled by $\alpha\beta APP_{444-592}$, as well as by αC7 (data not shown). A polyclonal antibody (1280) to synthetic $A\beta P_{1-40}$ and a monoclonal antibody (6C6) to synthetic $A\beta P_{1-28}$ only weakly labeled the 22-kDa band and FL-βAPP (data not shown). This result is consistent with the often poor recognition by such peptide antibodies of the AβP region within FL-βAPP or fragments thereof (20).

In addition to the FL-βAPP and 22-kDa proteins, immunoblots of concentrated Q Sepharose eluates of microvessel detergent extracts revealed two bands of 11 kDa and 16 kDa specifically labeled by all our carboxyl-terminal antisera [e.g., αB6, αC7 (Fig. 3*C*)], but not by αB5 (Fig. 3*C*). The 11-kDa band comigrated with the previously described carboxyl-terminal fragment in transfected 293 cells that results from constitutive cleavage of FL-βAPP (7, 8).

Comparison of the 22-kDa βAPP Fragment Among Brain Regions, in Nonneural Tissues and in Other Species. As judged by immunoblotting, the 22-kDa protein was present in similar quantities in microvessel homogenates prepared from frontal, temporal, parietal, and occipital cortices of the same human brain (Fig. 4, lanes 1–4). Caudatoputamen microvessels contained amounts similar to cortex (Fig. 4, lane 5), but

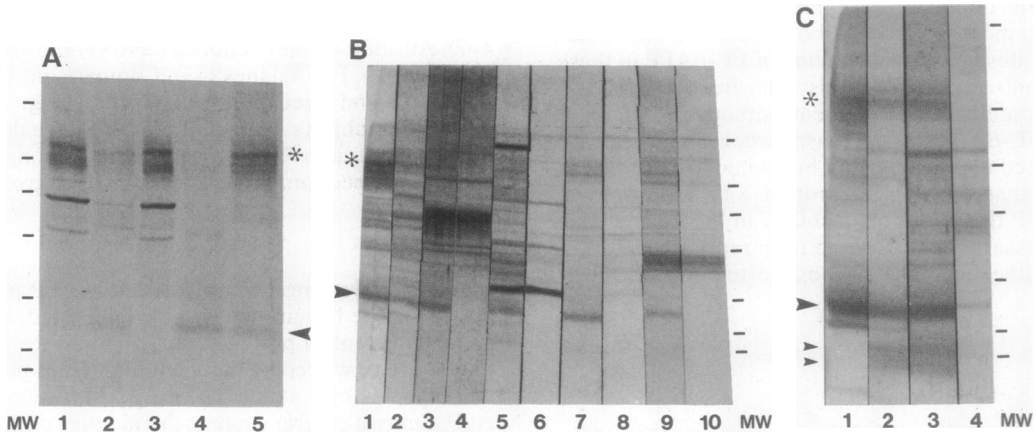

FIG. 3. (*A*) Immunoblot of various fractions from a human brain stained with αB5 (1:1500) (15 μg of protein per lane). The acrylamide gradient minigel was 5 to 20%. Lanes: 1, total homogenate of cerebral cortex; 2, purified cerebral cortical microvessels; 3, cerebral cortical homogenate lacking microvessels (flow-through fraction off 73-μm nylon mesh); 4, 1% Triton X-100 extract of cerebral cortical microvessels; and 5, Q Sepharose column eluate (0.4 M NaCl) of 1% Brij 35 extract of cerebral cortical microvessels. All fractions are from the same human brain (76-yr-old male with AD). The 22-kDa protein (arrowhead) is detectable only in microvessels and their extracts. The relatively weak staining of FL-βAPP (asterisk) in lane 4 may relate to the need to perform acetone precipitation solely on this sample to remove the Triton before electrophoresis. M_r (MW) markers are as in Fig. 2. (*B*) Immunoblot of a Q Sepharose column eluate (0.4 M NaCl) of the 1% Brij 35 extract of human cerebral cortical microvessels stained with various antibodies to regions of βAPP. Acrylamide gradient minigel was 5–20% (15 μg of protein per lane). Lanes: 1, αB5 ($\beta APP_{444-592}$) (1:1500); 2, αB6 ($\beta APP_{592-695}$) (1:300); 3, αC1 ($\beta APP_{676-695}$) (1:250); 4, αC1 absorbed with peptide C1 (25 μg of peptide per μl of serum); 5, αC4 ($\beta APP_{666-695}$) (1:750); 6, αC4 absorbed with peptide C4 (6 μg/μl); 7, αC7 ($\beta APP_{676-695}$) (1:750); 8, αC7 absorbed with peptide C1 (6 μg/μl); 9, αC8 ($\beta APP_{676-695}$)(1:500); and 10, αC8 absorbed with peptide C1 (15 μg/μl). In addition to the 110- to 135-kDa FL-βAPP proteins (asterisk), the 22-kDa protein (arrowhead) is specifically labeled by all antibodies. Anti-C2 ($\beta APP_{681-695}$) also stained the 22-kDa protein specifically, and monoclonal 1G5 ($\alpha\beta APP_{444-592}$) stained it weakly (data not shown). M_r (MW) markers are as in Fig. 2. (*C*) Immunoblot of a Q Sepharose column eluate (0.4 M NaCl) of the 1% Brij 35 extract of cerebral cortical microvessels. Acrylamide gradient minigel was 5–20% (40 μg of protein per lane). Lanes: 1, αB5 (1:1500); 2, αB6 (1:300); 3, αC7 (1:750); and 4, αC7 absorbed with synthetic peptide C1 (6 μg/μl). In addition to FL-βAPP (asterisk) and the 22-kDa protein (large arrowhead), proteins migrating at 11 and 16 kDa (small arrowheads) are specifically labeled by αB6 and αC7 but not αB5. M_r (MW) markers are as in Fig. 2.

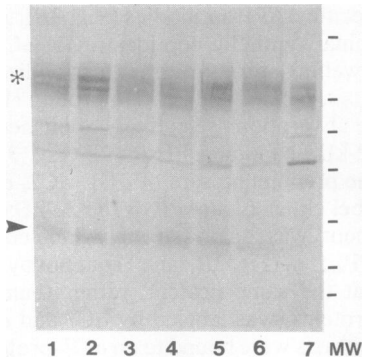

FIG. 4. Immunoblot of homogenates (6 μg of protein per lane) of purified cerebral microvessels from various regions of the same AD brain (an 87-yr-old male) stained with αB5 (1:1500). Acrylamide gradient minigel was 5–20%. Lanes represent microvessels from the following tissues: 1, frontal cortex; 2, temporal cortex; 3, parietal cortex; 4, occipital cortex; 5, caudatoputamen; 6, subcortical white matter; and 7, cerebellum. Asterisk, FL-βAPP proteins. Arrowhead, 22-kDa fragment. M_r (MW) markers are as in Fig. 2.

preparations from cerebellum or subcortical white matter usually showed smaller amounts, despite the presence of similar amounts of FL-βAPP in samples loaded for equal protein content (Fig. 4, lanes 6 and 7). The 22-kDa protein was readily detected in both small and large cortical microvessels (see *Materials and Methods*) but only weakly detected in small arteries of the arachnoid meninges and not detected in the middle meningeal artery (a dural branch of the external carotid artery), even when the latter two preparations were loaded at 10-fold higher protein concentration than cortical microvessels (data not shown). Microvessel preparations from renal cortex, heart, spleen and adrenal gland showed very little, o no, detectable 22-kDa protein despite the detection of FL-βAPP in these fractions (data not shown).

Cerebral microvessels prepared from rat and guinea pig showed very little, or no, 22-kDa band detectable by immunoblotting with our most sensitive carboxyl-terminal antibodies (αC7 and αB6), despite robust staining of FL-βAPP in these fractions. In contrast, microvessels from fresh bovine and rabbit brains contained the fragment, although its amount relative to the FL-βAPP was decreased when compared with fractions prepared identically from human cortex. To lessen the possibility that the 22-kDa protein was a breakdown product of βAPP that arose artifactually in the postmortem period, microvessels were prepared from rat brain and from bovine brain, either immediately postmortem or after incubating the tissue for 20 hr at room temperature. In the rat, no 22-kDa fragment was produced; in the cow, the fragment was present at 0 hr and 20 hr in equal amounts. In both species, the amount of FL-βAPP before and after the incubation did not differ (data not shown).

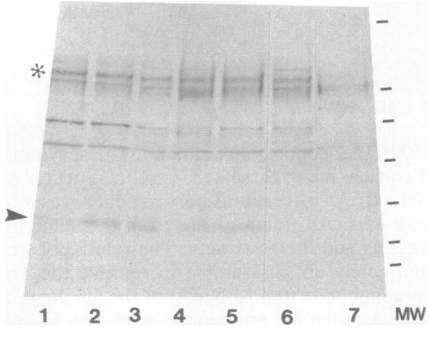

FIG. 5. Immunoblot of homogenates (5 μg of protein per lane) of cerebral cortical microvessels from non-AD brains ranging in age from fetus to 78 yr stained with αB5 (1:1500). Acrylamide gradient minigel was 5–20%. Lanes (postmortem intervals are in parentheses): 1, 78-yr-old male (M) (4 hr); 2, 47 M (19 hr); 3, 39 M (9 hr); 4, 3-mo M (12 hr); 5, 7-mo female (F) (18 hr); 6, 4-mo F (25 hr); and 7, 20-week fetus. Arrowhead, 22-kDa protein. Asterisk, FL-βAPP proteins. M_r (MW) markers are as in Fig. 2.

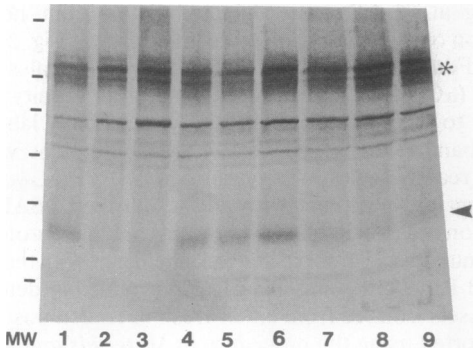

FIG. 6. Immunoblots of homogenates of cerebral cortical microvessels (15 μg of protein per lane) from AD and aged control brains stained with αB5 (1:1500). Acrylamide gradient minigels were 5–20%. Lanes: 1, AD [81-yr male (M)] with extensive amyloid angiopathy (AA); 2, AD (69 M) with extensive AA; 3, AD (65 M) with extensive AA; 4, AD (89 M) with mild AA; 5, AD (76 M) with moderate AA; 6, AD (81 M) with focal, mild AA; 7, normal (78 M); 8, normal (59 female); and 9, normal (72 M). Arrowhead, 22-kDa protein; asterisk, FL–βAPP proteins. M_r (MW) markers are as in Fig. 2.

Comparison of the 22-kDa Fragment in Normal, AD, and Down Syndrome Brain. Microvessels prepared from frozen cortical tissue of non-AD brains ranging in age from fetus to 78 yr were examined (Fig. 5). No consistent effect of postmortem interval (range 4–25 hr) on the amount of the 22-kDa protein was seen (compare, for example, lanes 1, 2, and 6 in Fig. 5). Incubation of cortex with a 4-hr postmortem interval for 20 hr at room temperature produced no change in the amounts of both the 22-kDa and FL-βAPP proteins in the microvessels. Fetal and infant brain microvessels contained very little, or no, detectable 22-kDa fragment (Fig. 5, lanes 4–7). All adult brains examined (≥40 yr) showed the fragment (for example, Fig. 5, lanes 1–3). Comparisons of preparations from AD and aged control subjects (Fig. 6) and Down syndrome subjects (data not shown) revealed the 22-kDa protein in all cases. The amount varied among cases, and no clear differences among the three patient groups emerged.

DISCUSSION

Progress in defining the pathways of βAPP proteolytic processing in the human brain has been limited, given the difficulties inherent to protein chemical analyses in postmortem tissue. Here, we report the identification in purified cerebral microvessels of a stable 22-kDa βAPP fragment containing AβP. Several criteria support the identity of this protein as a potentially amyloidogenic fragment of βAPP ending at its carboxyl terminus. (*i*) All six antibodies to carboxyl-terminal βAPP epitopes that we have examined produce specific, absorbable staining of the 22-kDa protein. These include antibodies to different carboxyl-terminal synthetic peptides as well as affinity-purified antibodies to recombinant $\beta APP_{592-695}$ that specifically label FL-βAPP in a wide variety of tissues and cells (7, 15–17). These antibodies label FL-βAPP in all our microvessel fractions containing the 22-kDa fragment. (*ii*) The 22-kDa protein is recognized by αB5, an affinity-purified antibody to recombinant $\beta APP_{444-592}$ (14). (*iii*) Antibodies to recombinant or synthetic peptides of the amino-terminal portion of βAPP (e.g., $\alpha\beta APP_{20-302}$) do not label the 22-kDa band, consistent with its size and carboxyl-terminal reactivity. (*iv*) The protein is membrane-associated and extractable in nonionic detergent, like FL-βAPP and the constitutive 11-kDa

carboxyl-terminal fragment. During chromatographic purification of the protein from microvessels, the 22-kDa fragment elutes virtually simultaneously with FL-βAPP (Fig. 3*A*, lane 5), indicating similar biochemical properties.

Several findings make it highly unlikely that the 22-kDa fragment arises artifactually during the postmortem period. (*i*) There is no correlation between the amount of the 22-kDa band and the length of the postmortem interval. For example, purified microvessels from a brain with only a 4-hr postmortem interval (Fig. 5, lane 1) show a definite 22-kDa band, whereas microvessels from a brain with a 25-hr postmortem period (Fig. 5, lane 6) contain no such band, despite virtually equal amounts of FL-βAPP. (*ii*) Microvessels isolated from rat brain immediately postmortem or after 20-hr room temperature incubation contain no immunoreactive 22-kDa protein and show no detectable change in FL-βAPP. (*iii*) Microvessels purified from fresh bovine brain contain the 22-kDa fragment, and its amount and that of FL-βAPP do not change after incubating the tissue for 20 hr. It is also unlikely that this fragment is generated during our microvessel purification because (*i*) the microvessel-free fraction obtained during this procedure contains abundant FL-βAPP but no 22-kDa band (Fig. 3*A*, lane 3); (*ii*) microvessel purification from infant brain yields no detectable 22-kDa fragment, despite the presence of abundant βAPP (Fig. 5); and (*iii*) microvessels isolated identically from rat brain have no 22-kDa protein but robust βAPP.

In addition to the 22-kDa protein, we have also detected immunoreactive proteins of 11 and 16 kDa with all six of our carboxyl-terminal antibodies in the detergent extract of microvessels. The 11-kDa band comigrates with the constitutive carboxyl-terminal fragment of βAPP found in transfected cells and probably represents this normally occurring nonamyloidogenic peptide. Further characterization of the 16-kDa protein is necessary to determine whether it contains the AβP region and, thus, could also be a potentially amyloidogenic intermediate. Detection of the 22-kDa fragment in cortical microvessels from normal and AD brains after about age 40 yr (Figs. 5 and 6) is consistent with the occurrence of β-amyloid fibrils in vessel walls of both normal and AD brains during aging. Factors in addition to the generation of this fragment (e.g., mutations in βAPP or other genes) would be needed to account for the greater vascular deposition of AβP usually observed in AD than normal aged brains. The identification of the 22-kDa protein predominantly in microvessels of adult brains is consistent with evidence that β-amyloidosis shows considerable analogy to certain systemic amyloidoses known to have a predilection for blood vessels and to originate from circulating or vascular precursor proteins (21). FL-βAPP is expressed in cerebral vessel walls in both endothelial and smooth muscle cells (22, 23) and is also found in circulating platelets (24–26) and lymphocytes (26, 27). We are now examining each of these cells as a source of the 22-kDa microvessel protein. The fragment could also be localized to vascular basement membranes, which are abundant in our preparations. AβP fibrils have been localized to capillary and arteriolar basement membranes in AD cortex (28–30). Amyloid fibrils in certain systemic amyloidoses are characteristically localized to vascular basement membranes (31), suggesting that proteases that process amyloidogenic precursors or macromolecules that promote fibrillogenesis are enriched in this structure. Further studies of the localization of the 22-kDa microvascular protein and its relative abundance in vessels that are rich or poor in β-amyloid deposits may establish whether this fragment is an amyloidogenic intermediate in the critical process of AβP formation during aging and in AD.

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