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Alternative, Non-secretase Processing of Alzheimer's β-Amyloid Precursor Protein during Apoptosis by Caspase-6 and -8*

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder. Although the pathogenesis of AD is unknown, it is widely accepted that AD is caused by extracellular accumulation of a neurotoxic peptide, known as Aβ. Mutations in the β-amyloid precursor protein (APP), from which Aβ arises by proteolysis, are associated with some forms of familial AD (FAD) and result in increased Aβ production. Two other FAD genes, presenilin-1 and -2, have also been shown to regulate Aβ production; however, studies examining the biological role of these FAD genes suggest an alternative theory for the pathogenesis of AD. In fact, all three genes have been shown to regulate programmed cell death, hinting at the possibility that dysregulation of apoptosis plays a primary role in causing neuronal loss in AD. In an attempt to reconcile these two hypotheses, we investigated APP processing during apoptosis and found that APP is processed by the cell death proteases caspase-6 and -8. APP is cleaved by caspases in the intracellular portion of the protein, in a site distinct from those processed by secretases. Moreover, it represents a general effect of apoptosis, because it occurs during cell death induced by several stimuli both in T cells and in neuronal cells.

Alzheimer's disease (AD),[1] a progressive neurodegenerative disorder of later life, is characterized by deposition of β-amyloid plaques, accumulation of intracellular neurofibrillary tangles, and neuronal cell loss (1). It is widely believed that this disease is caused by the extracellular accumulation of the aggregated amyloidogenic form of Aβ peptide (Aβ1–42). This peptide arises from the processing of β-amyloid precursor protein (APP) by still unknown proteases (secretases) (2). The recent discovery of three genes linked to familial, early-onset forms of AD (FAD) has brought further support to this theory. The first to be discovered was, in fact, APP, the protein from which Aβ derives (3). Moreover, mutations in APP associated with FAD are more efficiently processed by secretases and generate increased amounts of long Aβ (4). Two other FAD genes, the highly homologous multipass membrane proteins presenilin-1 (5) and presenilin-2 (6, 7) (PS-1 and PS-2), also regulate APP processing (8). Of more importance, mutations in presenilins linked to FAD all increased processing of APP and the formation of Aβ1–42 (9–11).

Recent studies focusing on the physiologic role of APP, PS-1, and PS-2 have shown that these FAD genes regulate apoptosis and also that AD-associated mutations result in enhanced proapoptotic activity of these molecules (12–18). Finally, PS-1 and PS-2 have been found to be cleaved during apoptosis by caspase-3 (19–21), a protease whose activity is essential for neuronal apoptosis (22). Together, these data suggest an alternative model for the pathogenesis of AD according to which AD is caused by dysregulation of programmed cell death (PCD) and enhanced susceptibility of neurons to apoptotic stimuli.

These two apparently contrasting theories need not be mutually exclusive. An attractive possibility is that APP processing might regulate or be regulated during PCD. In support for this, it has been shown that APP processing and Aβ formation were enhanced during neuronal apoptosis (23, 24). To determine how general this finding is, we studied the processing of APP during other forms of apoptosis. In this study, we show that APP is cleaved during apoptosis by the cell death proteases caspase-6 and -8. Further, apoptotic cleavage of APP is common to cell death initiated by different stimuli and occurs in both T and neuronal cells. This processing is distinct from that mediated by secretases because it occurs in the intracellular portion of the protein between Asp^{664} and Ala^{665}, COOH-terminal to the processing sites of the secretases. The finding that three FAD proteins are substrates of caspase strengthens the possibility that dysregulation of PCD could be involved in the pathogenesis of AD.

MATERIALS AND METHODS

Cloning and Mutagenesis—The cDNA expressing the 695-amino acid-long form of human APP was cloned in the mammalian expression vector pcDNA3 (Invitrogen), and mutant constructs were obtained using the Transformer site-directed mutagenesis system (CLONTECH, Palo Alto, CA). The cDNA encoding for the COOH-terminal fragment of APP after caspase cleavage (APPCcas, Ala^{665}-Asn^{695}) and APPβstub were obtained by PCR (primer sense Ccas: acgtggatccgccgccatggccgctgtcaccccag; primer antisense Ccas and βstub: acgtccgtctagattacttcttcagcatcaccaaggtg; primer sense βstub: cgcggatccgccgccatgctgcccggtttggcactgctcctgctggccgcctggacggctcgggcgctggaatgcagaattccgacatgac) and cloned as a *Bam*HI/*Xba*I fragment into pcDNA3. The identity of each construct was confirmed by sequencing using an ABI Prism 377 automatic sequencer (Perkin Elmer).

Cell Lines and Cell Transfections—Human T-cell Jurkat cells were grown in RPMI 1640 containing 10% fetal bovine serum (Biofluids), 2 mM glutamine, 25 nM β-mercaptoethanol, 10 μg/ml streptomycin, 10 μg/ml penicillin, and 10 μg/ml gentamicin (Life Technologies, Inc.). Murine neuronal N2A cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 10 μg/ml streptomycin, and 10 μg/ml penicillin.

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[1] The abbreviations used are: AD, Alzheimer's disease; FAD, familial Alzheimer's disease; APP, β-amyloid precursor protein; PCD, programmed cell death; PS, presenilin; APPNcas and APPCcas: NH_2-terminal and COOH-terminal fragments resulting from caspase cleavage of APP, respectively; Aβ, β-amyloid peptide; PARP, poly(ADP-ribose) polymerase; PIPES, 1,4-piperazinediethanesulfonic acid; CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonic acid.

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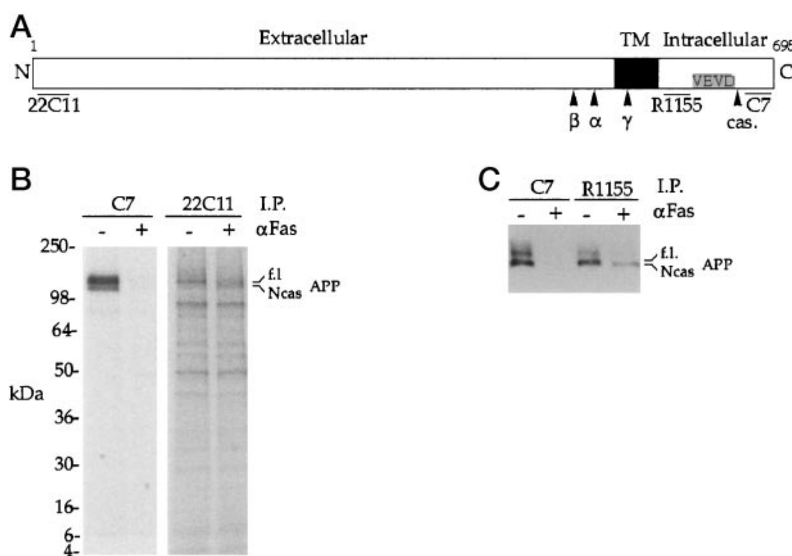

FIG. 1. **APP undergoes an alternative, non-secretase processing during apoptosis.** *A*, schematic representation of APP (numbering is based on the 695-amino acid isoform of human APP). The extracellular, transmembrane (TM, shown in *black*), and intracellular portions of the molecule are indicated. The cleavage sites for β-, α-, and γ-secretase and caspases (*cas.*) are indicated by *arrows*. The caspase consensus cleavage sequences present in the cytoplasmic tail (VEVD^{664}A^{665}), are marked. The regions recognized by the antibodies used in this study (22C11, R1155, and C7) are underlined. *B*, Jurkat cells were transfected with APP. Sixteen h after transfection, cells were metabolically labeled with [^{35}S]methionine. Two h later, half of the cells were induced to die with 100 ng/ml anti-Fas monoclonal antibody CH-11. After another 3 h, cells were lysed and culture medium was recovered. *In vivo* metabolically labeled APP was immunoprecipitated from both cell lysates and culture medium (not shown), gel separated, and revealed by autoradiography. Full-length APP (*f.l.*) is precipitated by the C7 antiserum only from the untreated sample (−). The 22C11 monoclonal specifically recognizes a smaller APP polypeptide (APPNcas) in the anti-Fas-stimulated sample (+). The multiple smaller bands observed in this immunoprecipitation with the 22C11 antibody are not recognized by this same reagent in western blot experiments (see Figs. 2*A*, 2*B*, and 3*A*), and are therefore likely to be aspecific. *C*, Jurkat cells were transfected with APP and treated the following day with anti-Fas antibody (CH-11). Cell lysates, prepared 6 h after the indicated treatments, were immunoprecipitated with either the C7 or R1155 antiserum, gel separated, blotted, and probed with the 22C11 antibody. Western blot analysis shows that APPNcas is precipitated only by the R1155 antiserum and possesses a lower molecular mass than APPf.l (86.9 kDa *versus* 83.2 kDa). Data shown in this figure were confirmed by three independent experiments. Bands of higher molecular mass than the full-length APP (see also Figs. 2, 3, and 5) represent posttranslationally modified APP species, derived from *O*-glycosylation, phosphorylation, and sulfation events occurring during APP trafficking in the cell (45).

Jurkat cells (4 × 10^6) (10^7/ml) were transfected by electroporation with 20 μg of the pcDNA3-APP construct. One h after transfection, dead cells were removed by Ficoll (Amersham Pharmacia Biotech) purification.

Anti-APP Antibodies—Regions of APP recognized by the antibodies used in this study are depicted in Fig. 1. R1155 and C7 are rabbit polyclonal antiserum raised to a synthetic polypeptide of amino acids 649–664 and 676–695 of APP (numbering according to Kang *et al.* (Ref. 25)), respectively (described by Selkoe *et al.* (Ref. 26) and Podlisny *et al.* (Ref. 27)). The monoclonal 22C11 antibody recognizes an N-terminal epitope of APP and is commercially available (Roche Molecular Biochemicals).

In Vivo Protein Labeling—Twenty-four h after transfection, Jurkat cells were washed once with methionine-free RPMI medium supplemented with 10% dialyzed fetal bovine serum (Life Technologies, Inc.) and incubated in the same medium for 40 min at 37 °C. ^{35}S-Labeled methionine (Amersham Pharmacia Biotech) (200 μCi) was then added in each transfection, and labeling continued for 2 h before the addition, where necessary, of anti-Fas.

Apoptosis Studies—Apoptosis was triggered in Jurkat cells 24 h after transfection by direct stimulation of the Fas molecule. Cells were supplied with fresh medium containing a monoclonal human anti-Fas antibody CH-11 (Upstate Biotechnologies, Inc.) at 0.1 μg/ml, incubated for the indicated time at 37 °C, and harvested. Alternatively, in Jurkat and N2A cells, apoptosis was stimulated with 250 ng/ml staurosporine (Sigma) for 6 to 10 h. The block of caspase-3 activity in Jurkat cells was obtained by incubating cells for 30 min with 50 μM ZDEVD-fmk, an irreversible caspase inhibitor (Enzyme System, Livermore, CA) prior to anti-Fas addition. Fifty μM mock inhibitor ZFA-fmk (Enzyme System) was used as a control. Of note, caspase-3 activity is required for caspase-6 activation (28).

Immunoprecipitation and Immunoblot Analysis—Cells were harvested, washed once in ice-cold phosphate-buffered saline, and lysed in lysis buffer (50 mM Tris, 150 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, pH 7.6) containing the protease inhibitors 4-(2-aminoethyl)-benzenesulfonyl fluoride hydrochloride, aprotinin, pepstatin, and leupeptin (Sigma). Lysate was spun at 10,000 × g for 10 min, and the supernatant recovered. Antibody was added and the immunoprecipitation reaction incubated at 4 °C for 12 h with gentle rocking. Immunocomplexes were captured by addition of protein A/G-agarose beads (Pierce) and incubation for 2 h at room temperature. Beads were washed three times in wash buffer I (50 mM Tris, 500 mM NaCl, 2 mM EDTA, 0.2% Nonidet P-40, pH 7.6), washed three times in wash buffer II (50 mM Tris, 150 mM NaCl, 2 mM EDTA, 0.2% Nonidet P-40, 0.1% SDS, pH 7.6), and resuspended in 100 μl of Laemmli loading buffer. Samples were heated at 95 °C for 2 min and separated on a polyacrylamide-SDS gel (Novex Experimental Technology). For detection of *in vitro* labeled proteins, gels were fixed, enhanced, vacuum-dried, and exposed to x-ray films at −80 °C using intensifying screens. Unlabeled proteins were blotted onto nitrocellulose membranes and probed with the specified antibodies. Immunoblots were developed using the SuperSignal system (Pierce).

In Vitro Cleavage Assay—[^{3}H]Leucine- or [^{35}S]methionine-labeled proteins were made using a TNT-coupled transcription and translation system (Promega, Madison, WI). Caspase cleavage reactions were performed at 37 °C in 25 μl of caspase buffer (20 mM PIPES, 100 mM NaCl, 10 mM dithiothreitol, 1 mM EDTA, 0.1% CHAPS, 10% sucrose, pH 7.2) containing 0.5 μg of recombinant caspase (PharMingen).

RESULTS

Alternative, Non-secretase APP Processing during Apoptosis—To determine whether increased APP processing and Aβ production was a general phenomenon associated with apoptosis, we studied APP processing during Fas-induced cell death in Jurkat T cells. To this end, Jurkat cells were transfected with a vector expressing APP and subjected to metabolic labeling with [^{35}S]methionine. Two h later, cells were treated with a cytotoxic anti-Fas antibody (CH-11) for 3 h. Cell lysates and cell culture supernatants were immunoprecipitated with either the 22C11 monoclonal antibody or C7 antiserum, specific for NH$_2$- and COOH-terminal regions of APP, respectively (Fig. 1*A*). Surprisingly, the C7 antiserum precipitated negligible levels of full-length APP from anti-Fas-treated cells (Fig. 1*B*,

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left panel). On the other hand, the anti-NH_2-terminal monoclonal antibody 22C11 revealed the presence of an APP polypeptide of lower molecular weight, termed APPNcas (Ncas), in lysates from cells undergoing apoptosis (Fig. 1*B*, *right panel*). In addition, the 22C11 monoclonal antibody did not precipitate any soluble APP proteins in the Fas-stimulated sample (data not shown). Together, these data indicate that the COOH-terminal region of APP containing the C7 epitope is cleaved off during apoptosis and that the NH_2-terminal APP fragment, APPNcas, remains associated with the cells.

The apoptotic processing of APP appears therefore to be distinct from that mediated by secretases, because the NH_2-terminal fragments resulting from these activities are usually released extracellularly. Alternatively, PCD could selectively enhance secretase processing of APP localized in intracellular compartments such as Golgi apparatus and endoplasmic reticulum. NH_2-terminal APP fragments generated by secretases in these compartments remain associated with the cells. To distinguish between these two possibilities, Jurkat cells were transfected with APP and treated with CH-11 the following day. After 6 h, cell lysates were immunoprecipitated with either C7 or R1155 antiserum. The latter is specific for an intracytoplasmic region of APP nested between the C7 epitope and the cleavage sites for β-, α-, and γ-secretases (Fig. 1*A*). Immunocomplexes were resolved by gel electrophoresis and probed with 22C11. As shown in Fig. 1*C*, the APPNcas molecule generated during PCD was immunoprecipitated by the R1155 but not by the C7 antiserum. Thus, APP is processed during PCD between the C7 epitope and the γ-secretase cleavage site.

APP Processing Is a General Phenomenon during Apoptosis and Requires Caspase Activity—Implementation of programmed cell death requires activation of caspases. These cysteine proteases are present in the cell as proenzymes and are activated by proteolysis. Active caspases execute cell death by cleaving intracellular substrates (29, 30). Thus, progression of apoptosis can be monitored by assessing the processing of caspases and their substrates. The 113-kDa protein PARP is one such substrate and is cleaved into two polypeptides of 89 and 24 kDa (31). Fas stimulation of APP-transfected cells induced apoptosis as shown by proteolytic activation of caspase-3 and cleavage of PARP (Fig. 2*A*, *two bottom panels*, *second lane*). Concomitantly, cleavage of APP and appearance of APPNcas was also detected (Fig. 2*A*, *top panel*). Thus, cleavage of APP correlates with activation of caspases.

To determine whether APP processing during apoptosis was dependent on caspase activity, transfected cells were treated with either an irreversible caspase inhibitor (ZDEVD-fmk) or with a control molecule (ZFA-fmk) prior to apoptosis induction. While ZFA-fmk had no effect, ZDEVD-fmk completely inhibited caspase-3 activation and, consequently, PARP cleavage (Fig. 2*A*, *two bottom panels*; compare *third* and *fourth lanes*). Consistently, inhibition of caspase activity resulted in suppression of the apoptotic processing of APP (Fig. 2*A*, *top panel*, *fourth lane*). Thus, cleavage of APP during PCD requires caspase activity.

To demonstrate that the apoptotic processing of APP is not specific to Fas-induced cell death but, rather, is common to apoptotic pathways initiated by several different stimuli, we treated Jurkat cells with staurosporine or ceramide, two other inducers of PCD. Both staurosporine (Fig. 2*B*) and ceramide (data not shown) resulted in the apoptotic processing of APP.

APP Is Cleaved by Caspases during Apoptosis between Asp^{664} and Ala^{665}—In looking at the amino acid sequence of APP, we noticed a putative caspase consensus cleavage sequence (Val^{661}-Glu-Val-Asp^{664}) (32–34) (Fig. 1*A*). This sequence is present in the cytoplasmic tail of APP, and cleavage at the predicted Asp^{664}-Ala^{665} site would give rise to an NH_2-terminal segment compatible in size with the APPNcas. Caspases have an absolute requirement for aspartic acid at position P1 of their substrate (32–34). To address whether APP was cleaved at this site by caspases during apoptosis, an APP mutant was made in which position P1 (Asp^{664}) was substituted with an asparagine (mutant D664N). As a control, another aspartate present in the extracellular portion of APP was mutated (mutant D620H). Jurkat cells were transfected with these two mutants, and apoptosis was induced with the anti-Fas antibody CH-11. While mutation of Asp^{620} did not affect the processing of APP, mutant D664N was completely resistant to cleavage (Fig. 3*A*, *top panel*). Of note, caspases were normally activated in these samples, as demonstrated by caspase-3 and PARP processing (Fig. 3*A*, *two bottom panels*). Identical results were also observed using an alternative approach. Transfected Jurkat cells were metabolically labeled, and APP was immunoprecipitated with the C7 antiserum or the 22C11 monoclonal antibody. As shown in Fig. 3*B*, APPNcas was specifically immunoprecipitated with the 22C11 antibody but not with the C7. Instead, mutation at the Asp^{664} completely abolished cleavage of APP by caspases. Together, these data indicate that APP is a direct substrate for caspases during PCD and that processing occurs at the Asp^{664}-Ala^{665} site of its cytoplasmic domain. Of interest, the APPCcas fragment generated in apoptotic cells is probably short-lived, because it could not be detected even when lysates from 4×10^7 cells taken at different time points after apoptosis triggering were analyzed by immunoprecipitation (data not shown).

APP Is Preferentially Cleaved by Caspase-6 and -8—Apoptosis progresses through a sequential activation of caspases. Triggering of Fas, for example, induces recruitment of caspase-8 to its intracytoplasmic tail followed by activation of this protease (35, 36). Active caspase-8 activates, either directly or indirectly, effector caspases such as caspase-3 and caspase-6. To test whether APP is directly cleaved by any of these caspases, we tested recombinant caspase-3, -8, and -6 in an *in vitro* cleavage assay using *in vitro*-translated recombinant APP protein as a substrate. As shown in Fig. 4*A* and

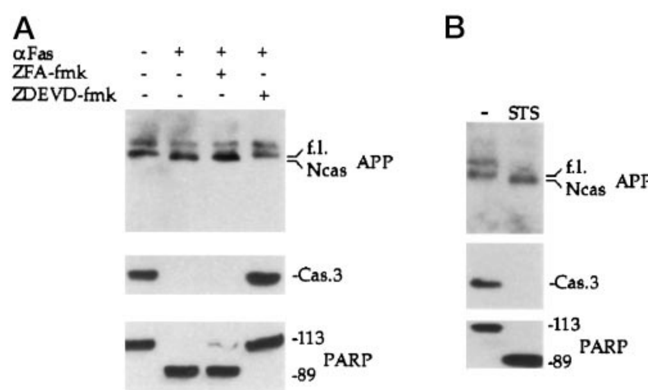

FIG. 2. **The alternative, non-secretase processing of APP is a general phenomenon during apoptosis and requires caspase activity.** *A*, cell lysates from APP-transfected Jurkat cells were prepared 6 h after the indicated treatments. Lysates were gel separated, blotted, and probed with the 22C11 antibody (*top panel*). Western blot analysis with anti-caspase-3 (*middle panel*) and anti-PARP (*lower panel*) antibodies showed that anti-Fas antibody induced proteolytic activation of caspase-3 (*Cas.3*) and cleavage of the endogenous substrate PARP. ZDEVD-fmk, a compound that blocks caspase activation and PARP cleavage, also inhibits APP processing. The irrelevant peptide ZFA-fmk has no effect on either caspase activation or APP processing. *B*, treatment of transfected Jurkat cells with 250 nM staurosporine (*STS*) for 10 h induces apoptosis as determined by caspase-3 activation, PARP cleavage, and APP processing. Data shown in this figure were confirmed by three independent experiments.

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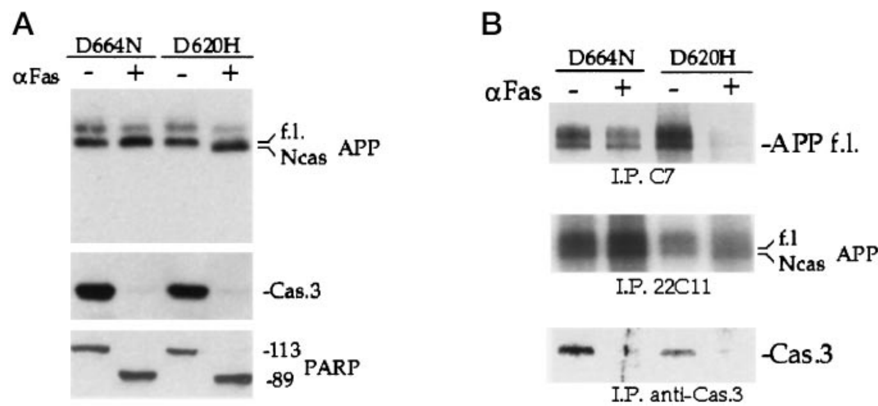

FIG. 3. **APP is directly cleaved by caspases between Asp[664] and Ala[665].** *A*, Jurkat cells were transfected with vectors expressing mutants D664N or D620H of APP. Cell lysates were analyzed for APP cleavage and caspase activation. Mutation of Asp[664] completely abolished APP cleavage without affecting caspase activation (*bottom panels*). *B*, cells transfected with the two APP mutants were metabolically labeled, immunoprecipitated with either C7, 22C11, or anti-caspase-3 antibodies, and analyzed as described in the legend of Fig. 1*B*. Also in this experimental setting, mutation of Asp[664] abolished caspase-mediated cleavage of APP. Data shown in this figure were confirmed by three independent experiments.

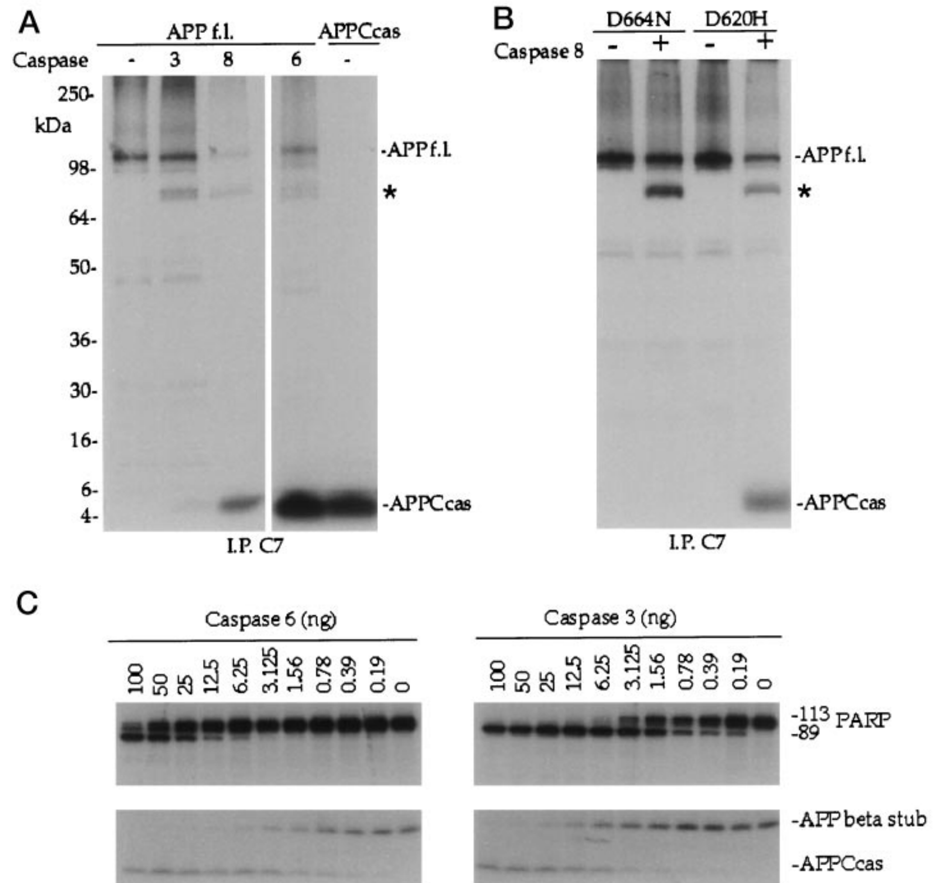

FIG. 4. **APP is cleaved by caspase-6 and -8 at the predicted Asp-Ala site.** *A*, *in vitro* metabolically labeled APP was incubated for 30 min at 37 °C with recombinant human caspase-3, -6, or -8, or caspase buffer (−). Samples were immunoprecipitated with the C7 antiserum and analyzed by gel electrophoresis. The full-length APP (*APPf.l.*) and the COOH-terminal APP cleavage product (*APPCcas*) are indicated. The *asterisk* points to an APP cleavage product that is not relevant because the cleavage site is contained in the extracellular portion of APP and therefore is not physiologically accessible to caspases during apoptosis (see also Figs. 2 and 3). *B*, mutation of Asp[664] completely abolished caspase-8-mediated APP cleavage. Data shown in this figure were confirmed by three independent experiments. *C*, comparison of PARP and APP cleavage by caspase-3 and -6 in a dose titration assay. *In vitro* metabolically labeled PARP, APPβstub, and APP (not shown) proteins were incubated for 1 h at 37 °C with the indicated amount of active recombinant human caspase-3 or -6. APP and APPβstub cleavage reaction were subsequently immunoprecipitated with the C7 antiserum, resolved onto a 10–20% tricine gel, and revealed by autoradiography.

recently by others (37, 38), APP was cleaved by caspase-3 and -8. However, both caspase-6 and -8 appeared to be more efficient in processing APP, as observed by the amount of the ~5 kDa fragment immunoprecipitated with the C7 antiserum (APPCcas; Fig. 4*A*). Thus, unlike PS-1 and PS-2, which are specific substrates of caspase-3 (18–20), APP *in vivo* might be preferentially cleaved by caspase-6 and -8. This conclusion is further substantiated by dose titration assays. In these experiments, caspase-3 cleaved more efficiently its established target PARP than APP, while caspase-6 showed the inverse specificity (Fig. 4*C*).

The size of the APPCcas fragment is compatible with processing occurring at the Asp[664]-Ala[665] site. This supposition was indirectly confirmed by the fact that *in vitro* transcription/translation of a construct encoding a polypeptide spanning from Ala[665] to the COOH terminus of APP (APPCcas) generated an APP polypeptide that co-migrated with APPCcas (Fig. 4*A*, *last lane*). However, to directly confirm this possibility, we produced recombinant-labeled APP D664N and D620H proteins and analyzed their susceptibility to caspase cleavage. As shown in Fig. 4*B*, while mutant D620H maintained APP sensitivity to caspase-8 processing, mutant D664N was resistant to it.

Caspase Processing of APP during Neuronal Cell Death— APP plays a central role in the etiological pathogenesis of both sporadic and familial forms of AD. Because this disease is

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FIG. 5. **Caspase-mediated cleavage of APP during neuronal cell apoptosis.** Apoptosis was induced in N2A neuronal cells by staurosporine (*STS*) treatment (250 ng/ml for 10 h). Triggering of apoptosis induced a shift in the gel mobility of APP detected in western blot analysis on total cell lysates (*T.L.*). This molecular mass reduction (3.7 kDa) is compatible with a caspase-mediated cleavage of APP. The APP polypeptide present in apoptotic neuronal cells is identical to the APPNcas molecule generated during T-cell apoptosis because it cannot be pulled down by the C7 antiserum but is readily immunoprecipitated (*I. P.*) by the R1155 antiserum. Data shown in this figure were confirmed by four independent experiments.

characterized by extensive neuronal loss, it was important to determine whether apoptotic processing of APP also occurred during neuronal cell death. To address this question, we used the mouse neuronal cell line N2A and induced PCD by treating these cells with staurosporine. Triggering of apoptosis resulted in a molecular shift of endogenous APP (Fig. 5, compare *first* and *second lanes*), similar to that observed for transfected APP in apoptotic Jurkat cells. Moreover, APPNcas, which could not be immunoprecipitated with the C7 antiserum (Fig. 5, *fourth lane*), was pulled down by the R1155 antiserum (Fig. 5, *sixth lane*). Thus, endogenous APP is readily and completely processed by caspases during neuronal apoptosis.

DISCUSSION

In the present study, we have demonstrated that APP is processed during apoptosis. Apoptotic cleavage of APP is common to death pathways initiated in diverse cell types and by various stimuli. The apoptotic proteolysis of APP is distinct from that mediated by secretases. In fact, while secretases cleave APP either in the extracellular (β- and α-secretases) or in the transmembranous (γ-secretase) regions of the protein (2), APP processing in apoptotic cells occurs in the intracytoplasmic tail. The intracellular portion of APP contains a canonical cleavage site for caspases. Using specific caspase inhibitors together with mutagenesis studies and an *in vitro* cleavage assay, we have shown that APP is a direct and specific substrate of caspase-6 and, to a lesser extent, caspase-3.

Caspases are the mammalian homologues of the cell death *Caenorhabditis elegans* gene *CED3*. *CED3* is essential for the execution of PCD in this nematode. Likewise, the proteolytic activities of caspases are required to complete the apoptotic program in mammalian cells. The proapoptotic function of these enzymes is mediated by processing of a number of intracellular substrates. Upon caspase-mediated cleavage, some endogenous proteins acquire proapoptotic activity, such as DFF/ICAD (39, 40), Bid (41), and procaspases themselves. Antiapoptotic proteins, such as Bcl-2, can also be processed by caspases, and this cleavage inactivates their protective function and results in the generation of proapoptotic polypeptides (42). Caspase cleavage has also been shown to generate a negative feedback signal. Processing of PS-2 by caspase-3 inactivates the proapoptotic function of PS-2 and generates a dominant negative inhibitor of apoptosis, PS2Ccas (21). Whether caspase-mediated cleavage of APP inactivates its proapoptotic role or, on the contrary, mediates the function of APP in cell death is currently under investigation in the laboratory.

Two neuronal proteins, FE65 and X11, have been shown to bind to the cytoplasmic region of APP (43, 44). Interestingly, the YENPTY motif of APP responsible for modulating this interaction is located COOH-terminal to the VEVD caspase cleavage site (43). It should then be concluded that the APPNcas fragment that originates from caspase cleavage does not bind to these two proteins. Therefore, upon caspase cleavage of APP the functional role of the APP/X11 and APP/FE65 interaction is likely to be lost. Thus, one possible immediate effect of caspase cleavage of APP is to abrogate its binding to X11 and FE65 and the functional consequences of these interactions. Whether APPCcas retains the ability to bind to X11 and/or FE65 and whether this putative interactions affect the progression of the apoptotic process remains to be determined.

Because AD is characterized by neuronal cell loss, one possibility is that the disease might be caused by dysregulation of PCD in neuronal cells. Studies regarding the biological functions of the three FAD genes have generated data supporting this hypothesis and lead to a model where even a slight imbalance in favor of apoptosis could eventually progress to neuronal cell loss seen in FAD. In fact, all three genes have been shown to positively regulate cell death, and FAD mutations generate molecules that enhance the sensitivity of cells to apoptotic stimuli (12–18). Moreover, all three FAD genes are acted on by the cell death proteases. The data we present here, together with the findings that apoptosis can enhance amyloidogenic processing of APP (22), raise the important possibility that cleavage of APP by caspases could regulate Aβ production. This link between apoptosis and production of amyloidogenic forms of Aβ could be relevant to the pathogenesis of AD. A correct understanding of the causative processes of AD represents an extremely important step in the identification of effective and much-needed therapeutic agents.

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