A growing body of evidence supports the notion that soluble oligomeric forms of the amyloid-β-peptide (Aβ) may be the proximate effectors of neuronal injuries and death in the early stages of Alzheimer disease. However, the molecular mechanisms associated with neuronal apoptosis induced by soluble Aβ remain to be elucidated. We recently demonstrated the involvement of an early reactive oxygen species-dependent perturbation of the microtubule network (Sonne, I., Fifre, A., Drouet, B., Klein, C., Koziel, V., Pincon-Raymond, M., Olivier, J.-L., Chambaz, J., and Pillot, T. (2003) J. Biol. Chem. 278, 3437–3445). Because microtubule-associated proteins (MAPs) are responsible for the polymerization, stabilization, and dynamics of the microtubule network, we investigated whether MAPs might represent the intracellular targets that would enable us to explain the microtubule perturbation involved in soluble Aβ-mediated neuronal apoptosis. The data presented here show that soluble Aβ oligomers induce a time-dependent degradation of MAP1A, MAP1B, and MAP2 involving a perturbation of Ca2+ homeostasis with subsequent calpain activation that, on its own, is sufficient to induce the proteolysis of isoforms MAP2a, MAP2b, and MAP2c. In contrast, MAP1A and MAP1B sequential proteolysis results from the Aβ-mediated activation of caspase-3 and calpain. The prevention of MAP1A, MAP1B, and MAP2 proteolysis by antioxidants highlights the early reactive oxygen species generation in the perturbation of the microtubule network induced by soluble Aβ. These data clearly demonstrate the impact of cytoskeletal perturbations on soluble Aβ-mediated cell death and support the notion of microtubule-stabilizing agents as effective Alzheimer disease drugs.

Microtubules are polymers of α- and β-tubulin dimers that mediate many functions in neurons, including organelle transport and cell shape establishment and maintenance as well as axonal elongation and growth cone steering in neurons. The polymerization, stabilization, and dynamic properties of microtubules are influenced by interactions with microtubule-associated proteins (MAPs). Members of this protein family are classified by size: high molecular mass proteins (MAP1A, MAP1B, MAP2a, and MAP2b) and intermediate molecular mass proteins (MAP2c, MAP2d, and tau) (1–3). Numerous studies have shown that neuronal apoptotic cell death involves alterations of the microtubule network consequent to calpain and effector caspase activation (4, 5). These calcium-dependent proteases are responsible for the degradation and turnover of a broad repertoire of MAP substrates, some of which they share, such as αII-spectrin and tau, and some of which are specific, as is the case of calpain-degraded MAP1B and MAP2.

Intraneuronal neurofibrillary tangles (NFTs) are one of the histopathological hallmarks in brains of patients diagnosed with Alzheimer disease (AD), a progressive dementia that manifests primarily as a profound inability to form new memories. These NFTs are composed of hyperphosphorylated tau organized into paired helical filaments (PHFs) (6). In addition, AD is also associated with the presence of extracellular senile plaques (7) formed as a consequence of the gradual accumulation and aggregation of the amyloid β-peptide (Aβ) into fibrils (8). Despite evidence that Aβ represents a key factor in AD (9), the nature of the toxic form of Aβ involved early in AD pathology remains to be determined. The issue of which pool (soluble or aggregated) of Aβ in brain is more deleterious in the early stages of AD is still controversial (10). However, clinicopathological hallmarks of AD correlate far better with the soluble pool of Aβ (11, 12). Moreover, several studies in transgenic mice have indicated that specific cognitive deficits, neurodegeneration, and synaptic loss might occur before any histologically detectable formation of senile plaques (13, 14). So, in reports from our group (15–18) and others (19, 20), attention has been paid to the soluble oligomeric forms of Aβ as the principal mediators of neurodegeneration in the early stages of AD development (10, 19–23).

Increasing evidence suggests that the selective neuronal cell death in AD involves activation of caspases, which critically participate in apoptosis through the initiation of intracellular pathways and the proteolytic cleavage of several target proteins responsible for the typical morphological changes of apoptosis (24, 25). Indeed, some experimental studies report the presence of activated forms of caspases and the accumulation of caspase-derived products in post-mortem AD brain tissue, similar to what has been observed in in vitro models of Aβ neurotoxicity (26–28). In addition, there is considerable evidence that an increased
activity of calpain associated with impaired calcium homeostasis (29, 30) may be involved in AD development (31, 32). A region-specific increase in calpain activity has been demonstrated in the early stages of AD development, even before synaptic loss, neuronal degeneration, and tau hyperphosphorylation and aggregation (33). Moreover, in AD brain, the active form of calpain co-localizes with NFTs, senile plaques, and neurpil threads (34), just like MAP1B and MAP2 (35, 36), which supports the capacity of MAP2 to form structures resembling PHFs via its microtubule-binding region (37). Moreover, MAP1 and MAP2 or their proteolytic products have been suggested to act as effectors of cell death in AD (1, 38). These observations strongly suggest the early involvement of caspases and calpains in the cytoskeletal disorganization and degeneration of neurons in AD (39).

We recently demonstrated that soluble Aβ oligomers induce neuronal apoptosis by biphasic modification of plasma membrane fluidity, leading to perturbation of the microtubule network that is dependent on the induction of early oxidative stress (17). We therefore tested the hypothesis that MAP modifications could be involved in the microtubule perturbation induced by soluble Aβ oligomers. The data presented here establish for the first time that calpain- and/or caspase-3-dependent proteolysis of MAP1A, MAP1B, and MAP2 could be a critical event involved in the microtubule disorganization and subsequent apoptosis induced by soluble Aβ in cortical neurons.

EXPERIMENTAL PROCEDURES

Materials—Aβ (1–40), Aβ (1–42), Aβ (40–1), caspase substrates (Ac-DEVD-7-amido-4-methylcoumarin (AMC) and Ac-LEHD-AMC), and inhibitor peptides (benzoylcarbonyl-VAL-fluoromethyl ketone (fmk), Ac-DEVD-fmk, and Ac-LEHD-chloromethyl ketone) were purchased from Bachem. Aβ (1–40) and Aβ (1–42) used in this study are synthetic peptides identical to the most predominant Aβ forms found in AD brains. Indeed, Aβ is a heterogeneous proteolytic fragment derived from sequential cleavage of the amyloid precursor protein by a variety of enzymes classically termed “secretases.” Initial cleavage of the amyloid precursor protein by β-secretase is followed by γ-secretase processing to release either the predominant 40-amino acid-long species (referred to here as Aβ (1–40)) or the slightly larger 42-amino acid-long variant (referred to here as Aβ (1–42)) according to the cleavage site along the amyloid precursor protein primary sequence. Aβ (40–1) is a synthetic peptide with the same amino acid composition and the reverse sequence of Aβ (1–40). Aβ (40–1) is widely used as a non-neurotoxic negative control. The LIVE/DEAD viability/cytotoxicity kit (catalog no. L-3224) and the calcium indicator Fluoro-3/AM (catalog no. F-1242) were purchased from Molecular Probes. The calpain substrate (succinyl-LY-AMC) and the calcium indicator Fluo-3/AM (catalog no. F-1242) were purchased from Molecular Probes. The calpain substrate (succinyl-LY-AMC) and the calcium indicator Fluo-3/AM (catalog no. F-1242) were purchased from Molecular Probes. The calpain substrate (succinyl-LY-AMC) and the calcium indicator Fluo-3/AM (catalog no. F-1242) were purchased from Molecular Probes. The calpain substrate (succinyl-LY-AMC) and the calcium indicator Fluo-3/AM (catalog no. F-1242) were purchased from Molecular Probes. The calpain substrate (succinyl-LY-AMC) and the calcium indicator Fluo-3/AM (catalog no. F-1242) were purchased from Molecular Probes. The calpain substrate (succinyl-LY-AMC) and the calcium indicator Fluo-3/AM (catalog no. F-1242) were purchased from Molecular Probes.

Neuronal Cell Culture Studies—Primary cultures of cerebral cortical neurons were prepared from embryonic day 15 C57BL/6J mouse fetuses as described previously (16). Briefly, dissociated cells were plated at 10^5 cells/cm^2 in plastic dishes or on glass coverslips precoated overnight with poly-DL-ornithine (1.5 or 15 μg/ml, respectively). The cells were cultured in chemically defined Dulbecco’s modified Eagle’s medium/nutrient mixture F-12 serum-free medium and supplemented with 500 nm insulin, 60 μM putrescine, 30 μM sodium selenite, 100 μM transferrin, 100 μM progesterone, and 0.1% (w/v) ovalbumin. Cultures were maintained at 35 °C in a humidified 6% CO_2 atmosphere. After 6–7 days in vitro, the cell population was determined to be at least 95% neuronal by immunostaining as described previously (16). Cortical neurons were treated at 6–7 days in vitro for the indicated times with soluble oligomers of Aβ (1–40) prepared as described (16). Alternatively, neurons were preincubated with different inhibitors or Ca^{2+}-chelating agent 2 h before and throughout the treatment with soluble Aβ oligomers.

Neuronal Viability and Apoptosis—Cell viability was initially determined by morphological observation. Aβ neurotoxicity was also assessed quantitatively by monitoring the mitochondrial reduction activity using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously (16). The simultaneous determination of live and dead cells was achieved with the LIVE/DEAD viability/cytotoxicity kit according to the manufacturer’s instructions.

To monitor apoptosis, cell nuclei were visualized using 4’,6-diamidino-2-phenylindole (DAPI). The neuronal cells (grown on glass coverslips) were washed with phosphate-buffered saline (PBS), fixed in PBS containing 4% paraformaldehyde for 30 min at room temperature, incubated for 10 min at room temperature with DAPI (0.1 μg/ml), washed with PBS, mounted in Fluoprep (bioMérieux S.A.), and visualized using a Leitz Aristoplan microscope equipped for fluorescence with a Fluotar ×40/1.3 objective. Photographs were taken using a Nikon DXM1200 digital camera. To evaluate the percentage of apoptotic cells, five independent fields of the microscope were counted (~200 cells) in three separate experiments, with three determinations in each experiment.

Measurement of Caspase-like Proteolytic Activities—The caspase activities were measured by cleavage of the substrates Ac-DEVD-AMC and Ac-LEHD-AMC as described previously (17). Briefly, at the indicated time points after Aβ treatment, the cells were washed three times with ice-cold PBS and incubated for 20 min on ice in buffer containing 25 mM Hepes (pH 7.5), 1% (v/v) Triton X-100, 5 mM EDTA, 1 mM EGTA, 5 mM MgCl_2, 5 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml each pepstatin and leupeptin, and 5 μg/ml aprotinin. After homogenization, the cells collected were then lysed by three cycles of freezing/thawing and centrifuged at 4 °C for 10 min at 10,000 × g, and the protein concentration was assayed using the BCA protein assay kit (Pierce). Fifty μg of proteins were incubated for 2 h with substrates (100 μM) initially dissolved at 10 μM in Me_SO. The cleavage of the caspase substrates was monitored by fluorescence emission at 460 nm after excitation at 360 nm using a Fluostar microplate reader (BMG LABTECH GmbH).

Measurement of Calpain-like Proteolytic Activity—The μM-calpain activity was measured by cleavage of the cyclic fluorogenic substrate succinyl-Leu-Tyr-AMC. Briefly, at the indicated time points following peptide treatment, cortical neurons were incubated for 1 h at 35 °C in a humidified 6% CO_2 atmosphere with 50 μM succinyl-LY-AMC. (The stock solution was 50 mM in Me_SO.) Following this, cells were washed with Hanks’ balanced saline solution, and fluorescence was directly recorded on culture dishes using the Fluostar microplate reader with 360-nm excitation and 460-nm emission filters.

Western Blot Analysis—Primary cortical neurons cultured under different conditions were washed with ice-cold PBS. Cells were then solubilized in 25 mM Tris-HCl (pH 7.4) lysis buffer containing 150 mM NaCl, 1 mM EDTA, 1% (w/v) sodium deoxycholate, 1% (v/v) Nonidet P-40, 0.1% (w/v) SDS, and protease inhibitors (Complete, Roche Applied Science). After homogenization, the cells collected were lyzed by three cycles of freezing/thawing and finally centrifuged at 4 °C for 10 min at 10,000 × g. The protein concentration in the supernatant was deter-
Time- and dose-dependent neuronal apoptosis induced by soluble Aβ oligomers. Primary cortical neurons were cultured for 24 h in the absence or presence of 5 μM soluble Aβ oligomers. Neuronal apoptosis is illustrated here with representative microscopic fields of phase-contrast micrographs (A), of the two-color fluorescence cell viability assay (LIVE/DEAD viability/cytotoxicity kit) (B), and of cell nuclei staining with DAPI (C). Aβ neurotoxicity was next determined by monitoring the mitochondrial reduction activity using the MTT assay (D). Cortical neurons were exposed to increasing concentrations of soluble Aβ (0.05–5 μM) for 24 or 48 h. Data are the means ± S.E. of three different experiments, with four determinations in each experiment, and are normalized to the effect of vehicle (designated as 100%). Differences between control and treated groups were analyzed using Student’s t test. ***p < 0.001.

Measurement of Intracellular Calcium Concentration—The measurement of cytosolic calcium concentration is based on the property of Fluo-3/AM exhibiting a large increase in fluorescence intensity without an accompanying spectral shift when binding Ca²⁺. An increase in fluorescence emission therefore reflects enhanced intracellular Ca²⁺ concentration. Briefly, at the indicated times, treated cortical neurons were loaded with 5 μM Fluo-3/AM (initially dissolved at 1 mM in Me₂SO) associated with Pluronic F-127 at 37 °C for 30 min. Cells were then washed with Hanks’ balanced saline solution, and fluorescence was directly recorded in culture dishes using the Fluostar microplate reader with 485-nm excitation and 530-nm emission filters.

For cytosolic Ca²⁺ imaging, the neuronal cells were grown on glass coverslips and subjected to the same protocol as described above. Cells were washed with PBS, fixed in PBS containing 4% paraformaldehyde for 30 min at room temperature, washed again with PBS, mounted in Fluoprep, and visualized under fluorescence microscopy.

Immunofluorescence Studies—Following treatment, neurons were fixed in PBS containing 4% (w/v) paraformaldehyde for 30 min at room temperature. The cells were permeabilized with PBS containing 3% bovine serum albumin and 0.1% (v/v) Triton X-100 for 30 min at room temperature and then incubated for 1 h at room temperature under constant shaking with monoclonal anti-β-tubulin antibody (1:500 dilution) or with goat polyclonal antibody against MAP1A (1:500 dilution), MAP1B (1:500 dilution), or MAP2 (1:500 dilution). After several washes with PBS, the cells were incubated for 1 h with TRITC-conjugated donkey anti-mouse IgG (Santa Cruz Biotechnology catalog number sc-12012; 1:500 dilution), or Alexa Fluor 488-conjugated donkey anti-goat antibody (1:1000 dilution), washed with PBS, labeled with DAPI as described above, and mounted in Fluoprep. The microtubules were visualized by fluorescence microscopy as described above.

Electron Microscopy—Cortical neurons were fixed for 2 h at 4 °C in 2.5% (w/v) glutaraldehyde and 0.5% (w/v) tannic acid in 0.1 M cacodylate buffer (pH 7.4). The neurons were then post-fixed for 2 h at 4 °C in 2% (w/v) osmic acid in phosphate buffer. After being dehydrated in a graded alcohol series, the samples were embedded in Epon resin (Poly/Bed 812, Polysciences, Inc.), and ultrathin sections (70 nm) were obtained using a Reichert Ultracut E ultramicrotome. Thin sections were counterstained with uranyl acetate and lead citrate prior to examination with a Jeol 100CX microscope.

Statistical Analyses—StatView computer software was used for statistical analysis. Data are from three separate experiments, with four determinations in each experiment, and are normalized to the effect of vehicle (designated as 100%). Values are expressed as the means ± S.E. Differences between control and treated groups were analyzed using Student’s t test. Multiple pairwise comparisons among the groups of data were performed using analysis of variance, followed by Scheffe’s post hoc test. Statistical differences were determined at p < 0.05. The
Western blot analysis results and fluorescent images shown are representative of at least three separate experiments.

RESULTS

Soluble Oligomers of Aβ Are Toxic to Mouse Cortical Neurons—We first investigated the neurotoxicity of non-fibrillar Aβ-(1–40) using primary cultures prepared from mouse cortex. Neuronal cultures incubated for 24 h with 5 μM soluble Aβ oligomers exhibited hallmarks of degenerating neurons, such as neurite alteration (Fig. 1A). The neurotoxicity of soluble Aβ was also investigated using a two-color fluorescence viability assay. In contrast to intense and uniform green fluorescence based on intracellular esterase activity displayed by control neurons, cells exposed for 24 h to 5 μM Aβ were stained mainly with ethidium homodimer-1, producing a bright red fluorescence indicative of dead neurons (Fig. 1B). Incubation of mouse cortical neurons with Aβ resulted in a time- and dose-dependent decrease in cell viability as monitored using the MTT assay (Fig. 1D). A 48-h exposure of cortical neurons to 0.5 and 5 μM Aβ induced a reduction in viability relative to controls (23.4 ± 3.0% (p < 0.001) and 77.4 ± 0.6% (p < 0.001), respectively). The decrease in neuronal viability upon Aβ treatment was significant after a 12-h incubation (11.8 ± 2.4% (p < 0.001) relative to controls at 0.5 μM) (data not shown) and reached levels of 58.2 ± 1.4% (p < 0.001) and 77.4 ± 0.6% (p < 0.001) after 24 and 48 h of treatment, respectively (Fig. 1D). Additionally, morphological examination of neuron nuclei stained with DAPI showed that cortical neurons exposed to soluble Aβ presented typical apoptotic morphology, with condensation of chromatin and fragmentation of the nuclei (Fig. 1C). Indeed, cortical neurons exposed to 2 and 5 μM Aβ for 24 h exhibited a dose-dependent increase in the number of apoptotic nuclei (38.2 ± 3.1 and 61.7 ± 4.9%, respectively; p < 0.001), whereas only 8.9 ± 2.8% of the control cells exhibited apoptotic nuclei (data not shown). We therefore selected a range of exposure times with 5 μM soluble Aβ to study the perturbation of the microtubule network and the associated intracellular events in the early stage of Aβ-induced apoptosis.

Soluble Oligomers of Aβ Induce Perturbation of Cellular Ca2+ Homeostasis—Cytosolic Ca2+ levels were first visualized using the fluorescent probe Fluo-3/AM. Labeling of control cells revealed weak cytoplasmic labeling, whereas cortical neurons exposed to 5 μM Aβ for 12 h exhibited increased fluorescence intensity, reflecting enhanced intracellular Ca2+ levels (Fig. 2A). As a positive control, a dramatic rise in cytosolic Ca2+ levels was noted after a 5-min treatment of cortical neurons with the Ca2+ ionophore ionomycin at 5 μM (Fig. 2A). The cytosolic Ca2+ content of cortical neurons was then quantified after treatment with 5 μM Aβ for increasing incubation times. The results revealed a time-dependent elevation of the cytosolic Ca2+ concentration (17.3 ± 2.7, 45.7 ± 3.5, and 77.5 ± 3.8% relative to controls at 3, 6, and 12 h, respectively; p < 0.001). Interestingly, Aβ-induced cytosolic Ca2+ influx was almost completely inhibited by addition of the Ca2+-chelating agent EGTA to the culture medium (increase of 77.5 ± 3.8% compared with 19.3 ± 4.1% relative to controls at 12 h; p < 0.05) (Fig. 2B). The positive control of the deregulation of Ca2+ homeostasis induced by 5 μM ionomycin for 5 min caused an increase of 610 ± 35% (p < 0.001) (data not shown). It is interesting to note that nifedipine, an inhibitor of cell membrane L-type Ca2+ channels, protected cortical neurons from soluble Aβ-mediated apoptosis. Altogether, these data suggest that a perturbation of cellular Ca2+ homeostasis is involved in the soluble Aβ-mediated neuronal death.

4 A. Fifre, I. Sponne, V. Koziel, B. Kriem, F. T. Yen-Potin, B. E. Bihain, J.-L. Olivier, T. Oster, and T. Pillot, unpublished data.
Soluble Aβ-induced MAP1A, MAP1B, and MAP2 Proteolysis

FIGURE 3. Calpain, caspase-9, and caspase-3 activation induced by soluble Aβ oligomers. Cortical neurons were incubated in the absence or presence of 5 μM Aβ for 12 h. Calpain, caspase-9, and caspase-3 activation was first monitored by measuring the proteolytic cleavage of the μ/m-calpain, caspase-9, and caspase-3 fluorogenic substrates (succinyl (Suc)-LY-AMC, Ac-LEHD-AMC, and Ac-DEVD-AMC, respectively) (A). Data are the means ± S.E. of three different experiments, with four determinations in each experiment, and are normalized to the effect of vehicle (designated as 100%). Differences between control and treated groups were analyzed using Student’s t test. ***, p < 0.001. Calpain and caspase-3 activation was next investigated by immunoblot analysis of all-spectrin breakdown product (SBDP) appearance, calpain regulatory subunit autolysis, and procaspase-3 processing of cortical neurons incubated in the absence (control (Ctl)) or presence of 5 μM Aβ for 12 h (B and C). Blots were stripped and reprobed with goat anti-β-tubulin polyclonal antibody.

Soluble Oligomers of Aβ Induce the Activation of μ- and/or m-calpain, Caspase-9, and Caspase-3—We next sought to determine whether soluble Aβ-induced apoptotic neuronal death is associated with the activation of μ- and/or m-calpain. Incubation of cortical neurons with 5 μM Aβ for 12 h increased the proteolytic cleavage of the μ/m-calpain-related substrate succinyl-LY-AMC (78.7 ± 8.1% relative to the control; p < 0.001) as shown in Fig. 3A. We next demonstrated that soluble Aβ also induced activation of the initiator caspase-9, which ensures the limited proteolysis of the effector caspase-3. Indeed, in the lysates of neurons exposed to 5 μM Aβ for 12 h, caspase-3 and caspase-9 activities increased to 83.8 ± 10.5 and 29.6 ± 1.1% (p < 0.001) relative to controls, respectively (Fig. 3A). Calpain and caspase-3 activation during the apoptotic process induced by soluble Aβ was also investigated by additional immunoblot analysis of the calpain regulatory subunit, αII-spectrin, and procaspase-3. The treatment of cortical neurons with 5 μM Aβ for 12 h demonstrated that the Ca2+-dependent autolysis of the small 28-kDa calpain regulatory subunit, generating the 18-kDa calpain regulatory subunit (Fig. 3B). It has been demonstrated that this common 18-kDa subunit associates with one of the two distinct autolyzed large subunits (76- and 78-kDa catalytic subunits) to form the typical activated μ- and m-calpains, respectively (40). These functional heterodimeric forms of calpain are able to cleave αII-spectrin, a major actin-binding cytoskeletal component. Indeed, incubation of cells with soluble Aβ generated calpain-related all-spectrin breakdown products, two fragments of 145 and 150 kDa (Fig. 3B, SBDPs 145/150 kDa). Aβ-induced caspase-3 activation was further demonstrated by generation of the active heterodimer composed of two p20 and two p17 subunits stemming from the full-length procaspase-3 precursor (Fig. 3C). Interestingly, the cleavage of αII-spectrin may also be a marker for caspase-3 activation (5). Indeed, immunoblot analysis of neurons exposed to 5 μM Aβ for 12 h demonstrated that αII-spectrin was also degraded to the caspase-3-related 120-kDa breakdown product (Fig. 3C, SBDP 120 kDa). Altogether, these data indicate that soluble Aβ oligomers induce activation of caspase-9, caspase-3, and calpain.

Soluble Aβ-induced Microtubule Fragmentation Involves Degradation of MAP1A, MAP1B, and MAP2—To investigate the kinetics of microtubule network disorganization, cortical neurons were incubated with 5 μM Aβ for 6 h. Under these conditions, treated neurons did not yet exhibit any detectable morphological features of apoptosis (compare Figs. 1 (A and B) and 4A). However, we observed a dramatic perturbation of the microtubule network in the majority of the neurites of treated neurons (as revealed by β-tubulin immunostaining) in comparison with control cells, whose microtubule network exhibited a dense and uniform labeling (Fig. 4A). Similar results were obtained using monoclonal
antibody against α-tubulin, the other monomer involved in the polymerization of the microtubule network (Fig. 4B). These immunocytochemical observations were confirmed by electron microscopy (Fig. 4C). Untreated cortical neurons showed a parallel and uniform organization of the elongated microtubule network within the neurites. Conversely, cells incubated with soluble Aβ for 6 h exhibited a microtubule disorganization characterized by short unparalleled microtubule segments and loss of microtubule continuity (Fig. 4C). We assessed the average length of the microtubules on the transmission electron micrographs (Fig. 4C), taking into account the following technical limitations as being part of electron microscopy. On the one hand, microtubules in control samples and, to a lesser extent, in treated cells were large enough to go off-scale most of the time. On the other hand, as ultrathin sections (70 nm) were required to visualize the microtubule network, the microtubules were virtually and necessarily interrupted because they processed over and under the longitudinal section. However, we determined that the microtubule fragments in cortical neurons exposed to 5 μM Aβ for 6 h averaged 1.1 ± 0.3 μm in length, which was significantly shorter than the microtubules in untreated cells, which were mostly longer than 3.7 ± 0.2 μm (p < 0.001).

We next investigated the role of structural MAPs in microtubule network disruption induced by soluble Aβ oligomers. Using immunocytochemistry, we examined both the protein levels and cellular distribution of neuritic MAP1A, MAP1B, and MAP2 isoforms (MAP2a, MAP2b, MAP2c, and MAP2d). It is worth noting that the distribution of MAPs in untreated cortical neurons is in keeping with that described in both in vivo and in vitro cell models (41). Indeed, MAP1A and MAP1B were more widely distributed in both dendrites and axons compared with the essentially dendritic processes and cell body localization of the four MAP2 isoforms. Untreated cortical neurons showed a normal MAP distribution, illustrated by a dense and uniform labeling (Fig. 5A). Conversely, neurons exposed to 5 μM Aβ for 12 h exhibited a loss of MAP labeling, particularly that located in the neurites (Fig. 5A). This disappearance of MAP immunostaining may reflect conformational changes or decreased levels of proteins, which was further confirmed by immunoblot analysis of MAP1A, MAP1B, and MAP2 levels (Fig. 5B). The treatment of cortical neurons with 5 μM Aβ for 12 and 24 h induced a time-dependent degradation of MAP1A and MAP1B, with the appearance of several degradation products located at ~75 kDa (Fig. 5B). In the absence of detectable variation of α- or β-tubulin subunits (Figs. 4 and 5B). Moreover, we did not detect any variation of the actin or glyceraldehyde-3-phosphate dehydrogenase signals (data not shown). Additionally, soluble Aβ induced a time-dependent degradation of both high molecular mass MAP2a and MAP2b as well as low molecular mass MAP2c (Fig. 5B). The degradation of both MAP2a and MAP2b was faster and more complete than that of MAP2c that was still present (although slightly decreased) after a 24-h Aβ treatment of neurons. It should be noted that, in our neuronal culture model, MAP2d is
inhibitors of calpain activity) prevented the degradation of MAP1A, MAP1B, MAP2a, MAP2b, and MAP2c induced by the treatment of neurons with 5 μM Aβ for 24 h (Fig. 7). In the case of MAP1A and MAP1B, the triplet of degradation products at ~75 kDa disappeared completely without fully recovering the amount of native proteins compared with untreated cells. Indeed, inhibition of calpain by EGTA or MDL28170 resulted in the production of an intermediate proteolytic fragment of ~250 kDa (Fig. 7A) that probably resulted from the cleavage of MAP1A and MAP1B by other protease(s). Interestingly, the formation of this intermediate cleavage fragment was completely inhibited in the presence of DEVD-fmk, an irreversible specific inhibitor of caspase-3. The treatment of neurons with 100 μM DEVD-fmk prevented MAP1A and MAP1B from being degraded, with a nearly complete recovery of the quantity of native proteins (Fig. 7A). However, the final proteolytic products were still faintly present, probably due to incomplete inhibition of caspase-3, associated with a preserved calpain activity (Fig. 7A). Finally, when calpain and caspase-3 inhibitors were used simultaneously, MAP1A and MAP1B degradation was almost completely inhibited, with the combined effects being greater than the individual effect of either MDL28170 or DEVD-fmk. This is further illustrated by the full recovery of the quantity of native proteins and the absence of intermediate and final proteolytic fragments. Similarly, having demonstrated that calpain and caspase-3 are responsible for MAP1A and MAP1B proteolysis, we also examined the involvement of these proteases in the degradation of the three MAP2 isoforms upon soluble Aβ treatment. The presence of EGTA and MDL28170 significantly abolished the proteolysis of MAP2a, MAP2b, and MAP2c (Fig. 7B). In contrast, the specific inhibition of caspase-3 by 100 μM DEVD-fmk had no effect on the Aβ-induced degradation of MAP2 (data not shown). Interestingly, exposure of cortical neurons for 24 h to the Ca2+ ionophore ionomycin (0.5 μM), which activates calpain, resulted in the degradation of the three MAP2 isoforms, similar to cells incubated with 5 μM Aβ (Fig. 7B). This effect of ionomycin on MAP2 was inhibited by the calpain-specific inhibitor MDL28170 (Fig. 7B). Similar results were obtained using another family of calpain inhibitors (N-protected tripeptide aldehyde) such as calpain inhibitors I and II (N-acetyl-Leu-Leu-norleucinal and N-acetyl-Leu-Leu-Met, respectively) instead of MDL28170 and a pan-caspase inhibitor (benzoyloxycarbonyl-VAD-fmk) instead of the caspase-3 inhibitor (data not shown).

**Soluble Aβ-induced Proteolysis of MAP1A, MAP1B, and MAP2 Is Oxidative Stress-dependent**—In a previous report, we demonstrated that soluble Aβ oligomers induce neuronal apoptosis via an early increase in the formation of reactive oxygen species preceding the Aβ-mediated microtubule perturbation (17). To characterize the involvement of oxidative stress in Aβ-induced MAP degradation, cortical neurons were preincubated with Trolox, a cell-permeable and water-soluble derivative of α-tocopherol. Preincubation of neurons with 1 mM Trolox prior to soluble Aβ exposure induced a persistent increase in cell survival as monitored by the MTT assay (61.1 ± 2.3% compared with 28.2 ± 3.2% reduction relative to controls; p < 0.05) (Fig. 8A). We thus investigated the effect of Trolox on the soluble Aβ-induced degradation of MAP1A, MAP1B, and MAP2. Immunoblot analysis showed that 1 mM Trolox partially prevented the degradation of MAP1A, MAP1B, MAP2a, MAP2b, and MAP2c induced by treatment of cortical neurons with 5 μM Aβ for 24 h (Fig. 8B). These data were assessed by densitometric analysis, and the results show that Trolox incubation significantly reduced Aβ-induced MAP cleavage as well as generation of degradation products (Table 1). Taken together, these results indicate that soluble Aβ-(1–40) oligomers induce a reactive oxy-

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**Figure 6. Specificity of the degradation of MAP1A, MAP1B, and three MAP2 isoforms involved in neuronal apoptosis induced by soluble Aβ oligomers.** The degradation of MAP1A, MAP1B, and three MAP2 isoforms induced by the two most common forms of the Aβ peptide and the reverse Aβ peptide was studied by immunoblot analysis of cortical neurons incubated in the absence or presence of Aβ-(1–40), Aβ-(1–42), or Aβ-(40–1) at 5 μM for 24 h. Alternatively, we also monitored the effects of the microtubule destabilizing agent nocodazole incubated for 1 h at 0.5 μM. Blots were stripped and reprobed with goat anti-β-tubulin polyclonal antibody.

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Expressed at very low levels compared with the three other isoforms. Therefore, measuring its possible degradation is beyond the sensitivity of the assay used in this study.

We next investigated the effect of soluble Aβ-(1–42), known to induce a more severe form of neurotoxicity, on MAP1A, MAP1B, and three MAP2 isoforms (Fig. 6). The treatment of cortical neurons with 5 μM Aβ-(1–42) for 24 h induced a more critical degradation of MAP1A, MAP1B, MAP2a, MAP2b, and MAP2c compared with 5 μM Aβ-(1–40). The degradation patterns were very similar in neurons treated with either Aβ-(1–40) or Aβ-(1–42), indicating that both soluble peptides induce neuronal apoptosis by involving the same pathways (16, 42). Moreover, the non-neurotoxic reverse peptide Aβ-(40–1) and the microtubule destabilizing molecule nocodazole did not lead to any degradation of MAP1A, MAP1B, or the three MAP2 isoforms, demonstrating that the degradation of these MAPs is specific to neuronal apoptosis induced by soluble oligomers of both Aβ-(1–40) and Aβ-(1–42) (Fig. 6).

**Calpain and/or Caspase-3 Mediates Soluble Aβ-dependent Proteolysis of MAP1A, MAP1B, and MAP2**—We further tested the hypothesis that calpain and/or caspase-3 might be involved in the soluble Aβ-induced degradation of MAP1A, MAP1B, and three MAP2 isoforms (MAP2a, MAP2b, and MAP2c) by monitoring the sensitivity of their degradation to different specific and nonspecific inhibitors. Immunoblot analysis showed that 1 mM EGTA as well as 10 μM MDL28170 (two
FIGURE 7. Synergistic involvement of calpain and caspase-3 in Aβ-dependent MAP1A, MAP1B, and MAP2 degradation. The involvement of calpain and caspase-3 activation in MAP degradation was monitored by immunoblot analysis of cortical neurons incubated in the absence or presence of 5 μM Aβ or 0.5 μM ionomycin for 24 h. The influence of calpain and caspase-3 inhibitors and Ca²⁺-chelating agent was investigated, in the case of MAP1A and MAP1B proteolysis (A), by incubating cortical neurons with 10 μM calpain inhibitor III (MDL28170), 100 μM DEVD-fmk, or 1 mM EGTA. Alternatively, in the case of proteolysis of MAP2a, MAP2b, and MAP2c (B), this involvement was investigated by incubating cortical neurons with 10 μM MDL28170 or 1 mM EGTA before and throughout the treatment with soluble Aβ. Blots were stripped and reprobed with goat anti-β-tubulin polyclonal antibody. We have verified that cells treated with the inhibitors only exhibited the same MAP profiles as control cells (data not shown).

FIGURE 8. Partial inhibition of Aβ-dependent MAP1A, MAP1B, and MAP2 proteolysis by Trolox. Cortical neurons were exposed to 5 μM Aβ2 for 24 h. The protective effect of Trolox on Aβ neurotoxicity was first determined by monitoring the mitochondrial reduction activity using the MTT assay (A). Data are the means ± S.E. of three different experiments, with four determinations in each experiment, and are normalized to the effect of vehicle (designated as 100%). ***, p ≤ 0.001. Statistical differences among the subgroups for each condition were performed by analysis of variance, followed by Scheffe’s post hoc test. #, p < 0.05. No significant differences were found between Trolox-treated and control cells. The effect of Trolox on MAP proteolysis was next monitored by immunoblot analysis of MAP1A, MAP1B, and three MAP2 isoform levels in primary cortical neurons incubated in the absence (control [Ctl]) or presence of 5 μM Aβ for 24 h (B). Cortical neurons were incubated with 1 mM Trolox before and throughout the treatment with soluble Aβ prior to immunoblot analysis. Blots were stripped and reprobed with goat anti-β-tubulin polyclonal antibody. We have verified that Trolox-treated and control cells exhibited the same MAP profiles (data not shown).
A

Taxol prevents the early reactive oxygen species-dependent cytoskeletal rodegeneration in AD (46). In support of this, we recently reported that observed in AD, leading to the idea of using microtubule-stabilizing genesis. For instance, studies using pharmacologic agents known to lead to neuronal apoptosis. Additional arguments favor the impairment of the microtubule system as a critical event associated with AD patho-

neurodegeneration and activation of several caspases (caspase-3 and -9) preceding and inducing the perturbation of the microtubule network, a process that is likely to contribute to impairment of axonal transport of various vesicles and organelles (43) such as mitochondria. This could explain the synaptic dysfunction observed in early clinical indications of AD or caused by soluble oligomeric assemblies of Aβ (44). Moreover, alterations of the microtubule network could affect its involvement in the spatial organization of the factors playing a role in signal transduction (45) that would eventually lead to neuronal apoptosis. Additional arguments favor the impairment of the microtubule system as a critical event associated with AD pathogenesis. For instance, studies using pharmacologic agents known to disrupt microtubules have reproduced some of the abnormalities observed in AD, leading to the idea of using microtubule-stabilizing drugs such as Taxol as potential therapeutic agents to slow down neu-

The results presented in this study provide evidence that the proteolysis of several structural MAPs is directly involved in the disruption of the microtubule network induced in the neuronal apoptosis induced by soluble oligomers of both Aβ(1–40) and Aβ(1–42). Indeed, soluble Aβ-mediated apoptosis involved an early perturbation of Ca²⁺ homeostasis with subsequent calpain activation. Calpain activation alone was required for the proteolysis of the three isoforms MAP2a, MAP2b, and MAP2c, whereas the Aβ-dependent activation of caspase-3 and calpain was responsible in a sequential manner for MAP1A and MAP1B proteolysis. By demonstrating that the proteolysis of MAP1A, MAP1B, and the three MAP2 isoforms was prevented by the presence of the antioxidant Trolox, we have confirmed the early involvement of reactive oxygen species generation and activation of several caspases (caspase-3 and -9ase) preceding and inducing the perturbation of the microtubule network induced by non-fibrillar Aβ (17).

The “amyloid cascade hypothesis” has been challenged in favor of an alternative hypothesis involving the more recently described soluble Aβ assemblies (10, 21–23), which could clarify many observations that cannot be fully explained, including the poor correlation between senile plaques and clinical symptoms of AD (13, 14). However, the molecular events associated with the cell death induced by soluble oligomers of Aβ remain only partially defined. It is of particular interest to note that the levels of soluble Aβ have been reported to directly correlate with NFT density (11). Furthermore, the severity of dementia and the extensive neuronal loss correlated far better with the number of NFTs than with the extent of Aβ deposition in the early stages of AD development (48). This would support the notion that modifications of the cytoskeleton of neurons might be involved early in the progression of AD pathology (49). Here, we have provided evidence that neurons exposed to soluble Aβ display a disruption of the microtubule architecture even before the typical morphological and biochemical alterations of apoptosis are detectable (Figs. 4 and 5A). A previous study has indicated that an obvious microtubule deficit can be seen in the absence of formation of PHFs in the early stages of AD development (50). Together with our data, these results therefore suggest that, besides the alteration of the structure and properties of tau (the most studied member of the MAP family), modifications of other members of this family (such as MAP1A, MAP1B, and MAP2) can contribute to the perturbation of the microtubule network in AD and ultimately lead to neuronal degeneration without accumulation of amyloid deposits.

Increasing evidence highlights the critical outcome of MAP modiﬁcation in cytoskeletal disorganization associated with the early stages of AD development. A decreased content of MAP1B and tau associated with cytoskeletal breakdown was found in the brains of AD patients compared with those of control individuals, suggesting a decreased capacity of microtubule assembly and stability (51). These results are consistent with those of Iqbal et al. (52) describing a decreased capacity in the in vivo microtubule assembly from brain extracts of AD patients. Moreover, the formation of neuroﬁlament threads involves a series of dendritic changes, including not only PHF accumulation, but also dramatic structural alterations such as microtubule perturbation and modiﬁcation and/or aggregation of several MAPs. One study has shown an early decrease in MAP2 labeling within dendrites from AD brain (53). In addition, accumulation of soluble Aβ oligomers in a transgenic mouse model of AD results in a decrease in the density of presynaptic terminals and in MAP2 labeling well before these mice develop plaques (13). Other studies have demonstrated that MAP1B and MAP2 co-localize with NFTs (35, 36). Despite the still unclear role of MAP1B and MAP2 in AD-associated neurodegeneration, several studies have highlighted the possible involvement of aberrant phosphorylation and proteolysis in several fragments (35, 54, 55), leading to MAP incorporation into PHFs as effectors of neurodegeneration in AD. Moreover, it has been demonstrated that small MAP2a and MAP2b fragments and MAP2c (via their microtubule-binding region) readily polymerize into structures resembling PHFs (37).

In this study, we have provided the first biochemical evidence that

**DISCUSSION**

| TABLE 1 | Densitometric analysis of Trolox inhibitory effect on MAP proteolysis induced by soluble Aβ |
|---------|------------------------------------------------------------------------------------------------|
| MAP1A   | Native protein | Cleavage products | Native protein | Cleavage products | MAP2a/b native protein | MAP2c native protein |
| Control | 100 ± 3        | 100 ± 13          | 100 ± 3        | 100 ± 13          | 100 ± 4              | 100 ± 2              |
| Aβ      | 73 ± 2*        | 190 ± 12*         | 60 ± 6*        | 194 ± 21*         | 64 ± 3*              | 73 ± 5*              |
| Trolox + Aβ | 86 ± 3*    | 143 ± 19          | 77 ± 3*        | 115 ± 6           | 80 ± 2*              | 87 ± 4*              |

*a p < 0.001.
*b p < 0.05.
*c p < 0.05.
*d p < 0.01.
depletion of functional MAP1A, MAP1B, and MAP2 induced by soluble Aβ might in part explain the disruption of the microtubule network during neuronal apoptosis (Figs. 5–7). We propose, as a mechanism of proteolysis, that the degradation of MAP1A and MAP1B is initiated by a caspase-3-dependent proteolytic cleavage to yield the 250-kDa fragments, whose accumulation might modify their properties and susceptibility to calpain proteolysis. The changes might include an altered conformation as well as abnormal interactions with other proteins or aggregation of MAP fragments. After being cleaved by caspase-3, these large MAP1A and MAP1B fragments can be further degraded by calpain into multiple smaller fragments. The pattern of limited proteolysis of MAP1A and MAP1B is in keeping with the fact that caspase-3 and calpain are known to generate fragments of limited size during the proteolysis of their substrates without further and complete degradation into very small peptides and amino acids (5, 40). However, it is possible that other caspases with shared specificity for caspase-3-sensitive sites in MAP1A and MAP1B contribute to their proteolysis and generate the intermediate fragment. Indeed, caspase-2 shares similarities with caspase-3 in their substrate preference and cleaves at the same DXD consensus sequence (56). We have eliminated this possibility by showing that a caspase-2 inhibitor (N-acetyl-Val-Val-Ala-aspartatal) used under the same conditions did not have any effect on MAP1A and MAP1B proteolysis (data not shown). Moreover, we have determined that calpain is not able to degrade full-length MAP1A and MAP1B if they have not been previously and independently cleaved by caspase-3. Indeed, treatment of cortical neurons with ionomycin increased the intracellular Ca2+ levels and subsequently activated calpain, but did not cause MAP1A and MAP1B degradation (data not shown). Calpain and caspase-3 interact in different ways to mediate limited proteolysis of a large number of proteins (4, 5, 40). This process can occur on the adenylate kinase (57, 58) or separate (57–60) calpain and caspase-3 cleavage sites along the primary sequence according to the stimulus (61) and depending on their cell type (62). It is also interesting to note that the degradation of tau (63) and huntingtin (64) occurs according to the same sequential process as described for MAP1A and MAP1B in this study, i.e. an initial degradation by caspase-3, followed by calpain proteolysis.

In this study, we have demonstrated that soluble Aβ induces a perturbation of Ca2+ homeostasis (Fig. 2). Several lines of evidence that strongly support a role for alteration of cellular Ca2+ homeostasis in the pathogenesis of AD come from studies of the effects of exogenously applied Aβ on cultured neurons (30) and from analysis of brain tissues from AD patients in association with neurodegenerative process (29). Interestingly, some investigators have suggested that Ca2+ dysfunction occurs during the initial phases of the disease, even before the development of overt symptoms and before any obvious extracellular Aβ deposits (65, 66). Consequently, destabilization of neuronal Ca2+ homeostasis might also be an important trigger of apoptosis via microtubule modifications (67) and activation of an array of cellular enzymes, including the calpain system (31). We have demonstrated here that exposure to soluble Aβ results in calpain-dependent proteolysis of the three isoforms MAP2a, MAP2b, and MAP2c (Fig. 7B). It has already been shown that MAP2a, MAP2b, and MAP2c are substrates for activated calpain (2). The calpain-dependent degradation of high molecular mass MAP2 is known to occur as an early intracellular structural event in response to traumatic (68), seizure-related (69), and focal ischemic (70, 71) brain injuries and to glutamate excitotoxicity (72). Similar MAP2 degradation is also associated with the proteolysis of MAP1A and MAP1B involving calpain activation in response to focal ischemic injuries (70) as well as in different in vivo and in vitro experimental models of neurotoxicity using various neurotoxic substances (73–76), in bipolar disorder (77), and in other neurodegenerative disease such as amyotrophic lateral sclerosis (78). However, it is noteworthy that the degradation of MAP2 is not associated with the proteolysis of MAP1B in the striatum or hippocampus after ischemia, whereas more extensive proteolysis of MAP2 and MAP1B occurs in other vulnerable brain areas (71). This discrepancy in the proteolysis of MAP2 and MAP1B depending on the brain area and/or the stimulus highlights that activated caspase-3 is necessary for the proteolysis of MAP1 family members.

Unlike caspase-3, which requires a specific amino acid sequence at cleavage sites (DXXD), calpain does not seem to have a strict sequence requirement for substrate cleavage, but rather recognizes secondary hydrophilic sequences called PEST sequences. These PEST sequences represent protein regions rich in proline, glutamate, aspartate, serine, and threonine. In addition to being recognition sites for calpain, such negatively charged clusters may bind Ca2+ and consequently locally activate calpain. It is even believed that the substrate specificity of calpain could be governed by the tertiary and quaternary structures of the polypeptide chain. The presence of accessible PEST sequences all along MAP1B, MAP2a, MAP2b, and MAP2c (as described by Friedrich and Aszodi (79)) has been established by several in vitro studies that described rapid proteolysis by the two typical purified μ- and m-calpains (80–83). Protein sequence analysis led us to identify a number of putative DXD cleavage sites for caspase-3 along the entire primary sequence of the structurally related proteins MAP1A and MAP1B. Furthermore, using the computer program PESTfind (84), we have observed that MAP1A and MAP1B contain a large number of PEST sequences.

Not only could MAP proteolysis result in loss of ability to promote the assembly and stabilization of microtubules, but also the MAP fragments could be toxic. Indeed, the cleavage affecting the tubulin-binding domain and the remaining two-thirds of MAP1A, MAP1B, and MAP2 representing the projection domain is likely to influence their association with microtubules, the spacing between microtubules, and their interactions with other cytoskeletal proteins or the plasma membrane (1, 2). It may also lead to negative regulation of MAP functions, known to provoke microtubule destabilization, modification of vesicle trafficking, and perturbation of signal transduction pathways. The hypothesis of loss of function may explain the association reported between estrogen-induced protection against Aβ-(1–40) neurotoxicity and increased expression of MAP1B and MAP2c (85). Caspase-3- and/or calpain-mediated MAP degradation and generation of several fragments may also contribute to the progression of soluble Aβ-induced neuronal cell death by release of proteolytic fragments that may exert a cytoskeletal disassembling function and/or pro-apoptotic effects. Indeed, caspase-3 cleavage products of tau were observed to induce apoptosis, turning tau itself into an effector of apoptosis (86, 87). Also, overexpression of N-terminal fragments of MAP1B has been shown to promote neuronal apoptosis (38).

We demonstrated previously that caspase-3 inhibition prevents neuronal apoptosis induced by soluble Aβ (17). In contrast, inhibition of calpain by several families of inhibitors (MDL28170, N-acetyl-Leu-Leu-Norleucinal, and N-acetyl-Leu-Leu-Met) had no effect on Aβ-induced apoptosis (data not shown). This suggests that caspase-3 activation is predominant and sufficient in MAP1A and MAP1B degradation and subsequent functional perturbations. Indeed, the sequential involvement of caspase-3 and calpain in the proteolysis of these MAPs would explain the complete recovery of the quantity of native proteins by inhibiting caspase-3, but not calpain (Fig. 7A). However, determining the relative roles of calpain and caspases in neuronal apoptosis is complicated by a growing body of evidence delineating a cross-talk between...
the two proteolytic systems (88). In this study, we demonstrated that Aβ-induced apoptosis required the activation of caspase-3 and caspase-9 by directly measuring caspase-like activities (Fig. 3A). Interestingly, calpain inhibitors further enhanced these proteolytic activities significantly even though Aβ-induced calpain activation was efficiently prevented (supplemental figure). The calpain-dependent cleavage of procaspase-3 has been shown to generate the active form and to lead to cell apoptosis (89). In contrast, activation of calpain may also prevent entry of cells into a caspase-dependent cell death program by inhibiting the activities of caspase-3 and caspase-9. Interestingly, the calpain-mediated truncation of procaspase-9 produces an inactive peptide that is unable to participate in cytochrome c- and dATP-induced caspase-3 activation (90–92). Another possibility is that the calpain-mediated proteolysis of procaspase-3 directly inhibits caspase-3 activation (91, 92). Additionally, the calpain-mediated proteolysis of active forms of caspase-3 and caspase-9 can be equally responsible for the down-regulation of activation (93). Moreover, in addition to direct cleavage of caspases, calpain has been reported to degrade several apoptotic regulatory proteins, including the apoptosome components apoptotic protease-activating factor-1 and Bax (94), indirectly inhibiting subsequent caspase-3 activation. Taken together, our results suggest that calpain may play a dual role during the time course of soluble Aβ-mediated neuronal apoptosis, negatively regulating caspase-9 and caspase-3 activation on the one hand and taking part in MAP degradation involved in the apoptotic process on the other.

In summary, this work identifies a novel mechanism associated with soluble Aβ-induced neuronal apoptosis. Our results reveal a direct relationship between Aβ exposure and the proteolysis of several MAPs, appearing to involve a caspase- and calpain-dependent pathway. Identification of this pathway may lead to the development of more effective therapeutic strategies for AD aimed at preventing soluble Aβ-mediated cytoskeletal breakdown and subsequent apoptosis.

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