Alzheimer disease (AD), the most frequent cause of dementia, is characterized by an important neuronal loss. A typical histological hallmark of AD is the extracellular deposition of β-amyloid peptide (Aβ), which is produced by the cleavage of the amyloid precursor protein (APP). Most of the gene mutations that segregate with the inherited forms of AD result in increasing the ratio of Aβ42/Aβ40 production. Aβ42 also accumulates in neurons of AD patients. Altogether, these data strongly suggest that the neuronal production of Aβ42 is a critical event in AD, but the intraneuronal Aβ42 toxicity has never been demonstrated. Here, we report that the long term expression of human APP in rat cortical neurons induces apoptosis. Although APP processing leads to production of extracellular Aβ1–40 and soluble APP, these extracellular derivatives do not induce neuronal death. On the contrary, neurons undergo apoptosis as soon as they accumulate intracellular Aβ1–40 and soluble APP. Here, we report that the long term expression of human APP in rat cortical neurons induces apoptosis. Although APP processing leads to production of extracellular Aβ1–40 and soluble APP, these extracellular derivatives do not induce neuronal death. On the contrary, neurons undergo apoptosis as soon as they accumulate intracellular Aβ1–40 and soluble APP. The Aβ peptide is a 39–43-amino acid peptide produced from a larger precursor, the amyloid precursor protein or APP (4). Among the ten identified isoforms of human APP (5), eight contain the Aβ sequence. The isoform that is mainly expressed in the human brain is a 695-amino acid protein known as APP695 (6). APP is processed by the non-amyloidogenic pathway, where α-secretase (7) and γ-secretase activities allow the release of Aβ. Several identified mutations in the APP and the presenilins genes segregate with inherited forms of AD known as early onset familial Alzheimer disease or FAD (8, 9). Most of these mutations result in an increased production of the Aβ ending at position 42 (10). In vitro studies have shown that Aβ42 rapidly aggregates into fibrils and that extracellular fibrillar Aβ peptides induce apoptosis in cultured neurons (11). On the other hand, recent reports have demonstrated an intraneuronal accumulation of Aβ42 in AD-vulnerable brain regions (12, 13). Intraneuronal Aβ42 accumulation has also been reported in transgenic mice expressing FAD proteins (14) as well as in transgenic mice showing accelerated neurodegeneration without extracellular amyloid deposition (15). Altogether, these data support the idea that Aβ42 accumulation and neuronal loss are closely correlated. Nevertheless, the direct link between Aβ production by neurons and neuronal death has not been clearly established until now.

Here, we report that the long term expression of human APP in rat-cultured neurons induces apoptosis. To understand how APP expression and processing modify neuronal survival, we characterized the extracellular and intraneuronal Aβ isoforms produced by the processing of different APP constructs. We further demonstrated that APP-induced neuronal apoptosis depends on intraneuronal Aβ1–42 accumulation.

Experimental Procedures

Cell Cultures and Reagents—Primary cultures of cortical neurons were prepared from 17-day-old Wistar rat embryos as described previously (16). Cells were plated in 6- or 96-well culture dishes (4 × 10^5 cells/cm^2) or glass coverslips (1.25 × 10^5 cells/cm^2) pretreated with poly(lysine) (10 μg/ml in phosphate-buffered saline) and cultured for 6 days in vitro in NEUROBASAL™ medium supplemented with 2% B-27 and 0.5 mM L-glutamine prior to infection with recombinant adenoviruses. Under these conditions, neuronal cultures (up to 98% of neurons) display high differentiation and survival rates (17). Transfected CHO cell lines expressing human APP695 (18) were cultured in F12 medium containing 10% fetal calf serum for 48 h before the culture medium was collected. DAPT, a functional γ-secretase inhibitor (19), was kindly provided by Aventis Pharma.

Recombinant Adenoviruses and Neuronal Infection—The construction of recombinant adenoviruses encoding β-galactosidase (AdRSVβ-gal) has been described previously (20, 21). The pAdRSVPP695 vector was generated by subcloning the EcoRl-Sal fragment of pHM-GAPF695 (22) in a pAdRSV vector (20). The deletion of the APP 695 intracellular domain was generated by PCR amplification of the APP 695 sequence using a 5′ primer (5'-AACGAAGTTGAGCCTGTTGATG-3') and a 3′ primer (5'-GTCGACCATGACAGGTCGGAGT-3') to create a 695-amino acid protein known as APP695. APP is processed by the non-amyloidogenic pathway, where α-secretase (7) and γ-secretase activities allow the release of Aβ. Several identified mutations in the APP and the presenilins genes segregate with inherited forms of AD known as early onset familial Alzheimer disease or FAD (8, 9). Most of these mutations result in an increased production of the Aβ ending at position 42 (10). In vitro studies have shown that Aβ42 rapidly aggregates into fibrils and that extracellular fibrillar Aβ peptides induce apoptosis in cultured neurons (11). On the other hand, recent reports have demonstrated an intraneuronal accumulation of Aβ42 in AD-vulnerable brain regions (12, 13). Intraneuronal Aβ42 accumulation has also been reported in transgenic mice expressing FAD proteins (14) as well as in transgenic mice showing accelerated neurodegeneration without extracellular amyloid deposition (15). Altogether, these data support the idea that Aβ42 accumulation and neuronal loss are closely correlated. Nevertheless, the direct link between Aβ production by neurons and neuronal death has not been clearly established until now.

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replaced by fresh culture medium for 3–5 days. Under these conditions, at least 75% of neurons express the proteins encoded by recombinant adenoviruses (16).

**Survival Assays and Nuclear Staining—**Neuronal survival was measured by the colorimetric MTT assay as described previously (23). Neurons grown in 96-well culture dishes were incubated after infection for 2 h at 37°C in fresh culture medium containing 0.5 mg/ml MTT. Medium was removed, and dark blue crystals formed were dissolved by adding 100 μl/well of lysis solution (isopropyl alcohol/0.04 N HCl). Outer diameter was measured on a microplate reader (492 nm). For nuclear staining, cells were fixed (0.37% formaldehyde/0.2% glutaraldehyde in phosphate-buffered saline) and incubated for 30 min in the Hoechst 33342 dye (1 μg/ml): Nuclear morphology was analyzed under fluorescence microscopy at excitation/emission wavelengths of 350/450 nm.

**Protein Analysis by Western Blot—**Cell culture medium and cell lysates were analyzed by Western blot as described previously (16). Cell lysates (20 μg of protein) and culture medium (15 μl) were subjected to 10% SDS-PAGE and blotted onto nitrocellulose membrane, incubated overnight at 4°C, washed, and incubated with 1/10,000 anti-mouse Ig horseradish peroxidase-conjugated secondary antibody followed by ECL revelation.

**Immunoprecipitation and Quantification of Aβ Production—**Aβ production was monitored by immunoprecipitation of cell culture medium. The quantification of Aβ1–40 and Aβ1–42 isoforms was performed by ELISA. Culture medium was collected, treated with protease inhibitors (1 μg/ml peptatin, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride), and cleared by centrifugation (16,000 × g, 5 min, 4°C). One hundred μl of the supernatant was used for Aβ quantification by fluorescent sandwich ELISA according to the manufacturer’s instructions (BIO SOURCE, Camarillo, CA). Previous experiments showed that there is no crossreaction between Aβ1–40 and Aβ1–42 recognition. Fluorescence emission was measured at excitation/emission wavelengths of 485 nm/535 nm. Immunoprecipitation was performed on 1.5 ml of the remaining culture medium with 15 μl/ml anti-Aβ whole rabbit serum (16). The immunoprecipitate was analyzed by Western blot on a 4–12% Nupage® gel using the WO2 antibody. Aβ was extracted from cell lysates by a modification of the protocol described previously (25). Neuronal cultures (37°C) cells were scraped and pelleted in cold phosphate-buffered saline. Cell pellets were solubilized in 300 μl of formic acid (70%). Formic acid-solubilized cell pellets were cleared (16,000 × g, 5 min, 4°C) to remove cell debris, and supernatants were centrifuged at 21,000 × g, 4°C for 20 min. The supernatants were vacuum-dried, and the resulting pellet was resuspended in 1 ml of alkaline carbonate buffer (2% Na₂CO₃, 0.1 N NaOH) and centrifuged (16,000 × g, 3 min, 4°C). Protein concentration was measured on 50 μl of the resulting supernatant by using the BCA protein assay (Pierce). For immunoprecipitation, 800 μl of the supernatant was neutralized with 800 μl of 1 M Tris-HCl, pH 6.8, and diluted 1:3 in H₂O. Immunoprecipitation was performed as described above. For ELISA, 100 μl of the remaining supernatant was neutralized with 100 μl of 1 M Tris-HCl, pH 6.8, and diluted 1:3 in H₂O containing 10% fetal calf serum, 0.5% Triton X-100, and 0.5% Nonidet P-40 (final concentrations). Aβ1–40 and Aβ1–42 concentrations were measured by ELISA on 100 μl of neutralized extract.

**Statistical Analysis—**The number of samples (n) in each experimental condition is indicated in the figure legends. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Bonferroni’s multiple comparison post-test.

**RESULTS**

**Long Term Expression of Human APP in Rat Cortical Neurons Induces Apoptosis—**When rat cortical neurons are infected by AdRSVAPP or AdRSV β-gal, a maximal and stable production of APP and β-gal is observed at day 3 postinfection (16). APP expression and processing have been analyzed by using the human-specific WO2 antibody (24). Five days after infection by AdRSVAPP, high levels of soluble human APP (sAPP) and Aβ are detected in the culture medium (Fig. 1A). This indicates that human APP is efficiently processed through both non-amyloidogenic and amyloidogenic pathways in rat neurons. The extracellular Aβ was quantified in the culture medium of neurons expressing human APP (Fig. 1B). These neurons secrete about 100 pg/ml of Aβ1–40, but there is no detectable extracellular Aβ1–42 (not shown). In the same experimental conditions, the MTT survival assay shows that human APP expression induces neuronal death, whereas β-gal expression has no effect (Fig. 1C). Taken together, these results demonstrate that a 5-day expression and processing of human APP in rat cortical neurons exert strong neurotoxic effects.

To further study the mechanism of the neuronal death triggered by human APP expression, neurons were stained with the Hoechst 33423 nuclear dye. This morphological analysis of the nuclei allows us to discriminate between the surviving and the apoptotic cells that display high nuclear condensation or fragmentation (26). Nuclear morphology of neurons was analyzed by Hoechst 33423 staining of non-infected (upper panel), AdRSV β-gal (middle panel), and AdRSV APP (lower panel) infected neurons. The nuclear shape of surviving neurons (filled arrow) or apoptotic neurons (open arrow) is indicated. Scale bar = 150 μm. E, quantification of the morphological analysis. For each condition, 10 fields of 3 independent cultures were analyzed. Results are given as the percentage of apoptotic neurons per field (**, p < 0.01, as compared with control; n = 8). D, nuclear morphology analysis (Hoechst 33423 staining) of non-infected (upper panel), AdRSV β-gal (middle panel), and AdRSV APP (lower panel) infected neurons. The nuclear shape of surviving neurons (filled arrow) or apoptotic neurons (open arrow) is indicated. Scale bar = 150 μm. E, quantification of the morphological analysis. For each condition, 10 fields of 3 independent cultures were analyzed. Results are given as the percentage of apoptotic neurons per field (**, p < 0.01, as compared with non-infected (NI) control).

**APP-induced Neuronal Apoptosis Does Not Involve Extracellular APP and Aβ—**We next analyzed whether the extracellular secretion of APP and Aβ could be responsible for the neurotoxic effects observed. To that end, neurons were incubated in the culture medium of a transfected CHO cell line or neuronal cultures expressing human APP695. The analysis of these two conditioned media indicates that they contain similar amounts of sAPP but different amounts of Aβ (Fig. 2A). The quantification of Aβ shows that the CHO-conditioned medium contains almost 100-fold more Aβ1–40 than the neuronal-conditioned medium. In addition, Aβ1–42 is present in the CHO culture medium, whereas it is undetectable in the neuronal culture.

**FIG. 1. Expression and processing of human APP triggers apoptosis in rat neurons.** A, analysis of neuronal culture medium 5 days after infection by AdRSVAPP (APP) or AdRSV β-gal (β-gal). Western blot (upper panel) showing the accumulation of sAPP in the culture medium of APP-infected neurons (NI = non-infected) and Aβ immunoprecipitation (lower panel) of the same medium. B, quantification of Aβ production by ELISA. Under the sensitivity threshold of the test (15 pg/ml), Aβ is not detectable (-). Results are given as mean ± S.E., (n = 6); C, neuronal survival measured by MTT assay. Results (mean ± S.E.) are given as the percentage of survival as compared with non-infected control cultures (**, p < 0.01, as compared with control; n = 8). D, nuclear morphology analysis (Hoechst 33423 staining) of non-infected (upper panel), AdRSV β-gal (middle panel), and AdRSV APP (lower panel) infected neurons. The nuclear shape of surviving neurons (filled arrow) or apoptotic neurons (open arrow) is indicated. Scale bar = 150 μm. E, quantification of the morphological analysis. For each condition, 10 fields of 3 independent cultures were analyzed. Results are given as the percentage of apoptotic neurons per field (**, p < 0.01, as compared with non-infected (NI) control).
FIG. 2. Extracellular APP and Aβ do not modify neuronal survival. Culture medium from neurons infected by AdRSVAPP (APP neuron) or from a CHO cell line stably expressing APP855 (APP CHO) were used to treat control neurons prior to the survival assay (NI = culture medium of non-infected neurons). A, the presence of saAPP in the conditioned medium was analyzed, before treatment, by Western blot (upper panel), and the presence of Aβ was monitored by immunoprecipitation (lower panel). B, quantification by ELISA of the Aβ present in the culture medium before treatment (mean ± S.E., n = 3). C, neuronal survival measured 2 days after treatment (n = 8).

medium (Fig. 2B). The treatment of neurons with these conditioned media does not significantly modify neuronal survival (Fig. 2C). Altogether, these results demonstrate that, in our model, the neuronal apoptosis induced by human APP is not triggered by any APP derivative, including Aβ1–40 and Aβ1–42, secreted in the culture medium. This raises the hypothesis that intracellular APP derivatives could be responsible for its neurotoxic effects.

APP-induced Neuronal Apoptosis Does Not Involve the Intracellular C-terminal Domain of APP—A possible origin of APP-induced neuronal death could be related to the intracellular C-terminal domain of the protein, which has been demonstrated to induce apoptosis in other cellular models (27, 28). To test this hypothesis, neurons were infected with a recombinant adenovirus (AdRSVAPPΔC) encoding a human APP isoform deleted in the intracellular C terminus of the protein. Five days after infection by AdRSVAPPΔC, saAPP and Aβ were detected in the neuronal culture medium (Fig. 3A). Neurons expressing human APPΔC secrete about 50 pg/ml of Aβ1–40 (Fig. 3B). This corresponds to half of the concentration of extracellular Aβ produced following the expression of full-length APP (Fig. 1B). In the same experimental conditions, the MTT survival assay shows that human APPΔC expression induces neuronal death (Fig. 3C). Hoechst staining indicates that APPΔC, like APP, triggers neuronal apoptosis (not shown). Taken together, these results demonstrate that the intracellular C-terminal domain of human APP is not involved in the neuronal death observed.

Intracellular Aβ1–42 Accumulation Induces Neuronal Apoptosis—Since the neurotoxic effect of APP does not involve the intracellular domain of the protein, we investigated whether the accumulation of intraneuronal Aβ could trigger apoptosis. An important fraction of intracellular Aβ has been shown to be insoluble (29, 30). Therefore, cells were solubilized in 70% formic acid as described previously (28) to recover all the intraneuronal Aβ peptide. The analysis of formic acid-solubilized cell pellets after 3 days of infection by AdRSVAPP or AdRSVAPPΔC reveals a similar expression pattern of the intraneuronal human proteins, although APPΔC is detected in lower amounts as compared with APP (Fig. 4A). In the same experimental conditions, immunoprecipitation of cellular extracts show that intraneuronal Aβ is undetectable (Fig. 4A). The survival assay shows that, after 3 days of infection, neither APP nor APPΔC expression causes neurotoxic effects (Fig. 4C), indicating that the overexpression of different levels of APP or APPΔC per se does not induce any neurotoxicity. After 5 days of infection, neurons still express different amounts of APP or APPΔC, but they accumulate similar amounts of intraneuronal Aβ1–42. (Fig. 4B) In all these experiments, intraneuronal Aβ1–40 was not detectable (not shown). After 5 days, both APP and APPΔC induce a massive neuronal death, as compared with non-infected or β-gal-expressing neurons (Fig. 4D). Taken together, these results clearly establish that, in our model, APP-induced neuronal death takes place only when intraneuronal Aβ1–42 is detected.

To further demonstrate that intraneuronal Aβ1–42 accumulation leads to neuronal apoptosis, neurons expressing human APP were treated with DAPT, a functional γ-secretase inhibitor (19). In our experimental conditions, DAPT does not display significant neurotoxicity by itself (not shown). Although DAPT treatment does not modify the secretion of saAPP in the culture medium, it reduces the extracellular Aβ1–40 concentration to a non-detectable level (Fig. 5A). DAPT also strongly reduces (57%) the production of intraneuronal Aβ1–42 without affecting the levels of APP expression (Fig. 5B). This reduction of intraneuronal Aβ1–42 production is concomitant with a significant recovery (52%) of cell survival (Fig. 5C). Altogether, these results demonstrate that the neuronal apoptosis induced by human APP in our model is triggered by the production and accumulation of intraneuronal Aβ1–42.

DISCUSSION

It is currently well admitted that APP plays a central role in AD, but less is known about the link existing between APP processing and the massive neuronal death that takes place in the disease. In the present study, we report that long term expression of human APP triggers apoptosis in rat neurons.
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This neuronal apoptosis is not related to the adeno viral-mediated overexpression of an exogenous protein since the adeno viral-mediated expression of β-galactosidase is without effect on neuronal survival. Our results are in line with previous studies showing that the adenoviral expression of human APP695 induces apoptosis in both rat hippocampal neurons (31) and rat brain (32). It is thus very important to understand how human APP could induce neuronal death.

We first investigated the role of secreted Aβ in APP-induced neurotoxicity. We utilized the culture medium of a CHO cell line or neurons expressing different levels of human APP as a source of extracellular Aβ. The concentrations of Aβ1–40 and Aβ1–42 in the CHO culture medium are comparable with those measured in the cerebrospinal fluid of AD patients (24). These concentrations of extracellular Aβ do not induce any neurotoxicity, indicating that extracellular soluble Aβ peptides are not directly responsible for neurodegeneration. It has been demonstrated previously that extracellular Aβ must aggregate into fibrils to acquire neurotoxic properties (11). At micromolar concentrations, fibrillar Aβ provokes oxidative injuries followed by cell death in neuronal and glial cells (33, 34). This extracellular Aβ toxicity could be mediated by the interaction of fibrillar Aβ with APP present at the neuronal membrane (35). In the present study, even when neurons express human APP at their cell surface, the extracellular Aβ produced fails to induce neuronal death.

The fact that APP-induced apoptosis occurs independently of secreted APP derivatives led us to investigate the role of the C-terminal domain of the protein in neuronal death. The APP C terminus is essential for the cell surface APP signaling function (36) and for the APP-dependent axonal anterograde transport (37, 38). Here we show that the neuronal expression of either C-terminal deleted APP (APPΔC) or full-length APP (APP695) induces apoptosis. In other cellular models, the C-terminal domain of APP has been shown to mediate cytotoxic effects. The cleavage of the intracellular domain of APP by caspases generates a C31 cytotoxic fragment in mouse N2a neuroblastoma cell lines (27). The interaction of the intracellular domain of the V642I APP mutant with G proteins leads to nucleosomal DNA fragmentation in F11 neuronal cell lines (28, 39). Since the neuronal apoptosis observed in this study is not mediated by the C-terminal domain of APP, we conclude that important differences in the metabolism and function of APP may exist between neuronal primary cultures and cell lines.

Another possible origin of APP-induced apoptosis is the intracellular accumulation of Aβ. Neurons have been shown previously to produce intracellular Aβ42 (25, 40). Here we report that neurons expressing human APP or APPΔC accumulate very similar amounts of intraneuronal Aβ1–42, whereas they produce different amounts of extracellular Aβ1–40. The extracellular Aβ production by neurons expressing APP or APPΔC is in agreement with previous observations in transfected cells (18, 41). After 3 days of infection, the expression of membrane human APP or APPΔC at different levels does not induce any neurotoxicity, indicating that the overexpression of APP per se is not toxic. In addition, the amount of intraneuronal Aβ1–42 is probably too low to be detected, and there is no neuronal damage observed. After 5 days of infection, the intraneuronal accumulation of Aβ1–42 in neurons expressing APP or APPΔC provokes a massive neuronal apoptosis. To further demonstrate that intraneuronal Aβ1–42 accumulation induces neuronal apoptosis, the intraneuronal production of Aβ1–42 was inhibited by a functional γ-secretase inhibitor, DAPT (19). DAPT was chosen among other functional γ-secretase inhibitors described (40) because it was the only one that was not neurotoxic by itself in our experimental conditions. Moreover, DAPT specifically inhibits Aβ production without affecting APP expression and processing through the non-amyloidogenic pathway. DAPT was able to reduce extracellular Aβ1–40 pro-

Fig. 4. APP-induced neuronal death is correlated with intraneuronal Aβ1–42 production. Neuronal cultures were infected by AdRSVβ-gal (β-gal), AdRSVAPP (APP), or AdRSVAPPΔC (APPΔC) for 3 days (3d) or 5 days (5d). A, Western blot of formic acid-solubilized cell pellets showing the presence of full-length intraneuronal APP (upper panel) and Aβ immunoprecipitation of the same cellular extracts (lower panel). B, quantification of intraneuronal Aβ1–42 production by ELISA (mean ± S.E., n = 4). C, neuronal survival measured by MTT assay 3 days after infection. Results are given as the percentage of neuronal survival as compared with non-infected control cultures (n = 12). D, neuronal survival measured by MTT assay 5 days after infection. Results are given as the percentage of neuronal survival as compared with non-infected control cultures (**, p < 0.001, as compared with control or with β-gal; n = 12).

Fig. 5. A γ-secretase inhibitor reduces intracellular Aβ production and restores neuronal survival. Neuronal cultures were treated for 5 days with 250 nM DAPT immediately after infection with AdRSVAPP (APP). A, accumulation of sAPP in the culture medium after 5 days of treatment analyzed by Western blot (top) and quantification of the extracellular Aβ release by ELISA (bottom) under the same conditions (mean ± S.E., n = 3). B, analysis of intraneuronal APP expression by Western blot (top) and quantification of Aβ accumulation (bottom) in formic acid-solubilized cell pellets (mean ± S.E., n = 6). C, neuronal survival measured by MTT assay 5 days after infection. Results are given as the percentage of neuronal survival as compared with non-infected (NI) control cultures (***, p < 0.001, ***, p < 0.01; n = 12).
duction to a non-detectable level and inhibited intraneuronal Aβ1–42 production by 57%. The differential inhibition observed at Aβ40 and Aβ42 sites could be viewed as evidence that different γ-secretases generate Aβ1–40 and Aβ1–42 or could result from the production of these two peptides in different cellular organelles to which γ-secretase inhibitors have access with different efficiency (42). The production of intraneuronal APP and Aβ was studied in three different experimental conditions: (i) production of APP without detectable Aβ1–42, (ii) production of APP and Aβ1–42, (iii) production of APP and partial inhibition of the production of Aβ1–42. Our results show that the DAPT-mediated inhibition of intraneuronal Aβ1–42 accumulation (57%) is similar to the DAPT-mediated recovery of neuronal survival (52%). This clearly indicates that the neuronal apoptosis that we observed results from the accumulation of intraneuronal Aβ1–42. The mechanisms by which intraneuronal Aβ accumulation triggers apoptosis are currently unknown. The highly amyloidogenic Aβ1–42 is produced in the endoplasmic reticulum/intermediate compartment of neuronal cells (43). The intracellular accumulation of Aβ1–42 may cause an overload of the endoplasmic reticulum, leading to neuronal cell injury (44).

Intraneuronal Aβ accumulation has been described in transgenic mice expressing FAD mutations. In double transgenic mice expressing human APP1 and PS1 mutants, intraneuronal Aβ accumulation precedes amyloid deposition (14). Intraneuronal Aβ42 accumulation together with extensive neuronal loss occurs without amyloid deposition in transgenic mice expressing a human PS1 mutation (15). Neuronal loss has also been documented in transgenic mice expressing the Swedish APP mutation that leads to plaque formation (45).

Intraneuronal accumulation of Aβ42 in AD brains has been recently reported (12). Although AD patients show a severe neuronal loss in specific brain regions, the involvement of apoptosis in AD neurodegeneration remains a matter of debate (46). Apoptotic features have been observed in brains of AD patients (47), but it may be very difficult to observe the transient apoptotic state of neurons when looking at the lesions several years after the onset of the disease.

In conclusion, our data, in agreement with other recent reports, strongly support the idea that the intraneuronal production and accumulation of Aβ1–42 are key events in AD. Elucidating how intraneuronal Aβ triggers apoptosis should in turn allow a better understanding of the neurodegeneration occurring in the disease.

Acknowledgements—We thank K. Beyreuther and L. Mercken for the generous gift of the W02 antibody and DAPT, respectively. Also, we acknowledge A. S. Caumont for the critical reading of the manuscript.
Intracellular Amyloid-β1–42, but Not Extracellular Soluble Amyloid-β Peptides, Induces Neuronal Apoptosis

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J. Biol. Chem. 2002, 277:15666-15670.
doi: 10.1074/jbc.M200887200 originally published online February 22, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M200887200

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