Production of Nerve Growth Factor by β-Amyloid-Stimulated Astrocytes Induces $p75^{NTR}$-Dependent Tau Hyperphosphorylation in Cultured Hippocampal Neurons

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Reactive astrocytes surround amyloid depositions and degenerating neurons in Alzheimer's disease (AD). It has been previously shown that β-amyloid peptide induces inflammatory-like responses in astrocytes, leading to neuronal pathology. Reactive astrocytes up-regulate nerve growth factor (NGF), which can modulate neuronal survival by signaling through TrkA or p75 neurotrophin receptor ($p75^{NTR}$). Here, we analyzed whether soluble Aβ peptide 25–35 (Aβ) stimulated astrocytic NGF expression, modulating the survival of cultured embryonic hippocampal neurons. Hippocampal astrocytes incubated with Aβ up-regulated NGF expression and release to the culture medium. Aβ-stimulated astrocytes increased tau phosphorylation and reduced the survival of cocultured hippocampal neurons. Neuronal death and tau phosphorylation were reproduced by conditioned media from Aβ-stimulated astrocytes and prevented by caspase inhibitors or blocking antibodies to NGF or $p75^{NTR}$. Moreover, exogenous NGF was sufficient to induce tau hyperphosphorylation and death of hippocampal neurons, a phenomenon that was potentiated by a low steady-state concentration of nitric oxide. Our findings show that Aβ-activated astrocytes potently stimulate NGF secretion, which in turn causes the death of p75-expressing hippocampal neurons, through a mechanism regulated by nitric oxide. These results suggest a potential role for astrocyte-derived NGF in the progression of AD.

Key words: Alzheimer's disease; apoptosis; astrocytes; NGF; p75 neurotrophin receptor

β-Amyloid peptides (Aβ), produced by cleavage of amyloid precursor protein, is the primary constituent of senile plaques in Alzheimer's disease (AD). Oligomeric or fibrillary forms of Aβ are cytotoxic in primary cultured neurons (Yankner et al., 1990; Loo et al., 1993; Pike et al., 1997) and stimulate glial reactivity (Pike et al., 1994; Bales et al., 1998; Hu et al., 1998; Bach et al., 2001; Sáez et al., 2004). Reactive astrocytes surround amyloid depositions and degenerating neurons in AD (Duffy et al., 1980; Schechter et al., 1981; Mancardi et al., 1983; Walker and Beach, 2002). However, the mechanisms by which reactive astrocytes influence neuronal survival remains controversial (Frederickson, 1992; Brunden and Frederickson, 2002; Walker and Beach, 2002). In cultured astrocytes, Aβ induces a reactive phenotype characterized by increased production of growth factors and inflammatory mediators, including interleukin (IL)-1β, IL-6, tumor necrosis factor-α, and nitric oxide (Pike et al., 1994; Bales et al., 1998; Hu et al., 1998; Akama and Van Eldik, 2000; Bach et al., 2001; Sáez et al., 2004), which may critically affect neuronal function and survival.

Nerve growth factor (NGF) modulates neuronal survival and differentiation through activation of the tyrosine kinase receptor TrkA, but it can also stimulate neuronal death by activation of p75 neurotrophin receptor ($p75^{NTR}$; Sneider, 1994; Majdan and Miller, 1999; Barrett, 2000; Miller and Kaplan, 2001; Chao, 2003; Nykjaer et al., 2005). NGF can be a mediator of tissue inflammation (Levi-Montalcini et al., 1996) and is up-regulated in neuropathologies characterized by prominent astrocytosis, such as AD

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(Crutcher et al., 1993; Scott et al., 1995; Fahnestock et al., 1996; Hock et al., 2000). Moreover, the expression of NGF receptors in neurons from cortex, hippocampus, and forebrain nucleus basalis is altered in AD. TrkA expression is reduced in early and late stages of AD (Boissiere et al., 1997; Mufson et al., 1997; Hock et al., 1998; Savaskan et al., 2000; Counts et al., 2004; Ginsberg et al., 2006), whereas $p75^{NTR}$ expression is not affected or is increased in damaged neurons from AD (Goedert et al., 1989; Ernfors et al., 1990; Mufson et al., 1992; Hu et al., 2002; Counts et al., 2004; Ginsberg et al., 2006). Moreover, normal aging is associated with a progressive increase in the level of $p75^{NTR}$ expression and a parallel decrease in the level of TrkA expression (Costantini et al., 2005). Several reports have shown that NGF signaling through $p75^{NTR}$ in the absence of TrkA induces apoptosis (Rabizadeh and Bredesen, 1994; Nykjaer et al., 2005). Therefore, the imbalance in the ratio of TrkA and $p75^{NTR}$ expression may result in increased NGF apoptotic signaling through $p75^{NTR}$.

We hypothesized that Aβ exerts indirect neurotoxic effects by inducing astrocytic NGF production, which may act in concert with other inflammatory mediators, such as nitric oxide (NO), to induce hippocampal neuron degeneration. In the present paper, we report that Aβ fragment 25–35 (designated here as Aβ) potently stimulated the expression and secretion of NGF in cultured astrocytes. Furthermore, astrocytic NGF stimulated tau hyperphosphorylation and hippocampal neuron death through $p75^{NTR}$. In addition, NO potentiated NGF-induced neuronal death, suggesting a cooperative noxious activity of these mediators on $p75^{NTR}$-expressing hippocampal neurons.

MATERIALS AND METHODS

Materials

Culture media and serum were obtained from Gibco BRL (Carlsbad, CA). Mouse NGF (2.5S) was obtained from Harlan (Madison, WI) and primers from Integrated DNA Technologies, Inc. (Coralville, IA). Blocking antibodies to NGF and $p75^{NTR}$ were from Chemicon (Temecula, CA). Monoclonal antibody to Alzheimer's hyperphosphorylated tau PHF1 was a generous donation of Dr. Peter Davis. All other reagents were from Sigma (St. Louis, MO) unless otherwise specified. Chemical reagents were of the highest analytical purity.

Cell Cultures

Primary astrocyte cultures were prepared from 1–2-day-old rat hippocampus according to the procedures of Saneto and De Vellis (1987), with minor modifications. Hippocampi were dissected and incubated with 0.25% trypsin-EDTA for 15 min at 37°C; afterward, tissue was washed with saline-HBSS solution and disgregated. Cells were collected by centrifugation, filtered through a 80-μm mesh cell dissociation sieve, and plated at a density of 1.5×10^6 cells per 25-cm^2 Nunc flask (Naperville, IL) in DMEM supplemented with 10% fetal bovine serum, HEPES (3.6 g/l), penicillin (100 IU/ml), and streptomycin (100 μg/ml). When confluent, cultures were shaken for 48 hr at 250 rpm at 37°C, incubated for another 48 hr with 10 μM cytosine arabinoside, and then amplified to 2×10^4 cells/cm^2 in a 35-mm Petri dish or glass coverslips. Astrocyte monolayers were >98% pure as determined by glial fibrillary acidic protein (GFAP) immunoreactivity and were devoid of OX42-positive microglial cells.

Hipoccampal neurons from 18E rats were prepared as described by Banker and Cowan (1977). Hippocampi were dissected and then dissociated following incubation in 0.25% trypsin-EDTA for 10 min at 37°C, then plated over poly-L-lysine (0.5 mg/ml) at a density of 5,000 cells/cm^2 for immunofluoresence and 15,000 cells/cm^2 for Western blots. Cultures were maintained for 3 hr in Neurobasal medium supplemented with 10% fetal bovine serum, and then the medium was replaced by N2-supplemented Neurobasal medium and maintained for 5 days. For coculture experiments, astrocytes plated over coverslips were mounted over hippocampal neurons grown in poly-L-lysine-coated 35-mm petri dishes.

Treatment of Astrocytes

Astrocyte monolayers were exposed to different concentrations of Aβ 25–35 soluble peptide (Sigma) in serum-free medium (Neurobasal suplemmented with N2). Control cultures received the same volume of vehicle (deionized distilled water). For cocultures, astrocytes were treated for 24 hr with Aβ, and, after extensive washing, astrocyte monolayers were mounted over neuronal cultures. The NO donor NOC-18 (DETA-NONOate; Alexis, San Diego, CA) was added directly to the culture media of 5 DIV hippocampal neurons (10 μM), and tau phosphorylation was assessed 24 hr later.

Western Blot

After extensive washing, cultured cells were homogenized in RIPA buffer with protease inhibitors, and homogenates were centrifuged at 15,000*g* for 10 min at 4°C. Protein concentration was quantified by the modified Bradford technique (Bio-Rad, Hercules, CA). Protein extracts from hippocampal neurons were separated by SDS-PAGE, electrotransferred to nitrocellulose membranes, and processed for immunodetection as described by Cross et al. (1993, 1996).

Immunofluorescence

Astrocyte monolayers seeded over covers were fixed with 4% paraformaldehyde, 4% sucrose in phosphate-buffered saline (PBS) at 4°C for 15 min, and permeabilized with Triton X-100 for 15 min. After blocking nonspecific binding with 2% bovine serum albumin (BSA) in PBS for 1 hr, samples were incubated with GFAP monoclonal antibody (1/400; Sigma). Cells were washed three times with PBS and incubated with secondary antibody coupled to FITC for 1 hr at room temperature. Nuclei were counterstained with DAPI (Molecular Probes-Invitrogen, Eugene, OR), and cells were examined under a Zeiss confocal microscope model LSM510 Meta and images processed.

Viability Assays

Neuronal survival was assessed by direct cell counting and MTT assay according with previous studies (Alvarez et al., 1999).

Determination of NGF Levels

Astrocytes monolayers were treated with Aβ under serum-free conditions, and NGF protein concentration in the culture medium was quantified by using the NGF Emax ImmunoAssay system kit (Promega, Madison, WI), following the manufacturer's instructions.

Tau Phosphorylation Patterns

Phosphorylation levels were evaluated by Western blot using antibodies specific for Alzheimer's epitopes: AT8 (Innogenetics) and PHF1 monoclonal antibody (Brandt et al., 1995; Alvarez et al., 2001).

Relative Quantitative RT-PCR for NGF

Total RNA was isolated with Trizol reagent (Gibco-BRL, Life Technology) and 2 μg of total RNA was randomly reverse transcribed with the RetroScript Kit (Ambion, Austin, TX). The levels of NGF mRNA were quantified by relative quantitative RT-PCR. The specific primer pairs for NGF (Promega) and QuantumRNA Classic 18S Internal Standards primers (Ambion) were used together in the reaction to coamplify the specific and the control amplicon. The PCRs were carried out in a 50-μl reaction volume containing 1 μl cDNA, 20 pmoles of each specific primer, 4 μl 18S primers (2:8 primer:competimer ratio), 200 μM dNTPs, 1.5 mM $MgCl_2$, 1.5 U Taq DNA polymerase, and 1× Taq DNA polymerase PCR buffer (Invitrogen). The cycling parameters were as follows: 94°C, 45 sec; 64°C, 45 sec; 72°C, 45 sec during 24 cycles, where amplification is in the linear range. Minus RT controls were included in each assay. The amplification products were run in nondenaturing 6% polyacrylamide gel and stained with Sybr Gold Nucleic Acid Gel Stain (Molecular Probes). Densitometric analysis was performed in NIH Image, and expression levels were normalized against the 18S levels.

Statistical Analysis

Data were analyzed using standard statistical packages (SigmaStat System, San Rafael, CA). All values are the mean of at least three independent experiments performed in duplicate. Comparison of the means was performed by one-way analysis of variance. Pairwise contrast between means utilized the Student-Newman-Keuls test, and differences were considered statistically significant at $P < 0.05$.

RESULTS

Aβ 25–35 Stimulates NGF Expression and Release in Hippocampal Astrocyte Cultures

Astrocyte monolayers became reactive when exposed to the Aβ active fragment 25–35 (Aβ), displaying the characteristic increased GFAP immunoreactivity and process development (Fig. 1A). Exposure of astrocytes to 5–25 μM Aβ did not overtly damage the monolayer as judged by phase-contrast microscopy and MTT assays (not shown). Moreover, Aβ peptide induced NGF mRNA expression in astrocytes in a dose-dependent manner (Fig. 1B). NGF mRNA levels increased by fivefold 24 hr after 15 μM Aβ treatment compared with vehicle. The increase in NGF mRNA expression was followed by a long-lasting accumulation of NGF into the culture medium (Fig. 1C). The concentration of NGF in the culture medium increased approximately 15-fold 24 hr after Aβ treatment (10 μM) and remained elevated for up to 72 hr (Fig. 1D).

Aβ-Activated Astrocytes Induce Tau Hyperphosphorylation in Hippocampal Neurons

We have previously shown that Aβ-activated astrocytes mediated tau hyperphosphorylation in cocultured hippocampal neurons by a mechanism involving nitric oxide production (Sáez et al., 2004). Astrocyte cultures previously treated for 24 hr with Aβ (10 μM) induced a four- to sixfold increase in neuronal tau hyperphosphorylation, as revealed by PHF1 and AT8 phosphorylation epitopes. Remarkably, this effect was partially abolished by blocking antibodies to NGF or $p75^{NTR}$ (Fig. 2A), suggesting an involvement of astrocytic NGF.

In agreement, conditioned media from Aβ-activated astrocytes reproduced tau hyperphosphorylation in pure hippocampal neuron cultures (Fig. 2B). This effect was entirely prevented by the addition of blocking antibodies to $p75^{NTR}$. In contrast, media from control astrocytes failed to induce tau hyperphosphorylation under identical experimental conditions. Remarkably, after addition of NGF (100 ng/ml) to the cocultures described above, nonstimulated astrocytes induced tau hyperphosphorylation in hippocampal neurons (Fig. 2B).

Aβ-Activated Astrocytes Induce $p75^{NTR}$-Dependent Apoptosis in Cocultured Hippocampal Neurons

We next analyzed whether Aβ altered the ability of astrocytes to maintain neuronal survival. Under control conditions, astrocyte monolayers allowed hippocampal neurons to survive and develop extensive neuritic processes. In contrast, astrocyte cultures previously treated for 24 hr with Aβ (10 μM) reduced neuronal survival by 50% over the next 72 hr (Fig. 3A). This effect was reproduced in pure hippocampal neuron cultures by conditioned media from Aβ-activated astrocytes (Fig. 3A). Blocking antibodies to NGF or $p75^{NTR}$ completely prevented neuronal loss induced by activated astrocytes (Fig. 3B), whereas nonimmune serum was devoid of effect (not shown). Moreover, the caspase inhibitors DEVD-fmk and VAD-fmk also prevented neuronal death, indicating the execution of an apoptotic mechanism.

Exogenous NGF and NO Can Induce Neuronal Death

We next determined whether the effect of activated astrocytes and their conditioned media could be reproduced by maintaining hippocampal neurons in the presence of exogenous NGF. NGF (100 ng/ml) reduced

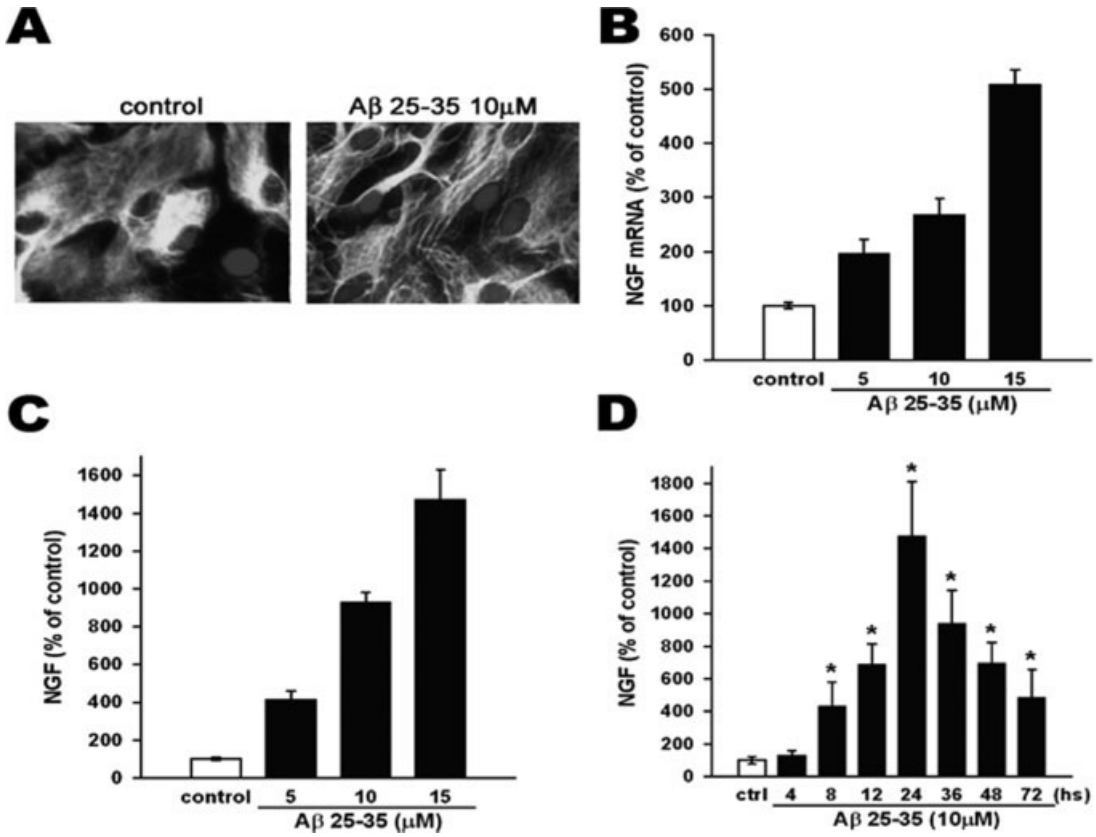

Fig. 1. Aβ 25–35 induces NGF expression and release in hippocampal astrocyte cultures. **A:** Fluorescence micrographs showing immunoreactivity for GFAP (green) in hippocampal astrocyte cultures 24 hr after exposure to 10 μM Aβ 25–35 or vehicle (control). **B:** Expression of NGF mRNA determined by RT-PCR of total RNA extracted at 24 hr after exposure to the indicated concentrations of Aβ 25–35 peptide. NGF mRNA levels are expressed as percentage of control. Data are mean ± SD of three independent RT-PCRs. All treatments are statistically significantly different from control ($P < 0.05$). **C:** NGF levels in the culture media of astrocytes treated with the indicated concentrations of Aβ 25–35 were assayed by ELISA. Data are express as percentage of NGF levels in control conditions (mean ± SD). **D:** Time course of NGF secretion after treatment with 10 μM Aβ 25–35. Data are express as percentage of NGF levels in control conditions (mean ± SD). $\star P = 0.05$ vs. control.

neuronal survival by ~20% after 72 hr (Fig. 4A). However, in agreement with our previous report (Pehar et al., 2004), the production of low steady-state concentrations of NO (<50 nM) from 10 μM NOC-18 potentiated neuronal death induced by NGF, reaching >50% after 72 hr (Fig. 4A). The flux of NO alone induced 30% of neuronal death. The effect of NGF, NO, and their combination on neuronal survival was coincident and correlated with tau hyperphosphorylation (Fig. 4B).

DISCUSSION

In the present study, we show an indirect neurotoxic effect of Aβ by activating astrocytes and inducing NGF production. Although NGF plays a key role in neuronal survival and differentiation through activation of the tyrosine kinase receptor TrkA, it can also stimulate neuronal death by activation of $p75^{NTR}$ (Sneider, 1994; Majdan and Miller, 1999; Barrett, 2000; Miller and Kaplan, 2001). $p75^{NTR}$ is not expressed in the adult hippocampus (Kiss et al., 1988), but it is highly expressed during the period of programmed cell death (Buck et al., 1988; Lu et al., 1989). Moreover, the ratio of TrkA/$p75^{NTR}$ expression is altered under conditions of damage (Roux et al., 1999) and in AD (Boissiere et al., 1997; Mufson et al., 1997; Hock et al., 1998; Savaskan et al., 2000; Hu et al., 2002; Counts et al., 2004; Ginsberg et al., 2006). In addition, in an in vivo model of hippocampal injury, p75 immunoreactivity colocalized with apoptotic neurons (Troy et al., 2002), suggesting its potential role in mediating elimination of damaged neurons. All the components of the neurotrophins family can induce death of cultured hippocampal neurons expressing $p75^{NTR}$ but lacking the cognate Trk receptor (Friedman, 2000). Therefore, induction of NGF by neighboring reactive astrocytes that occur under pathological conditions may render hippocampal neurons vulnerable to NGF-induced apoptosis. We provide evidence that Aβ potently stimulates NGF expression and secretion in astrocytes in concentrations sufficient to stimulate p75-dependent apoptosis in cocultured hippocampal neurons.

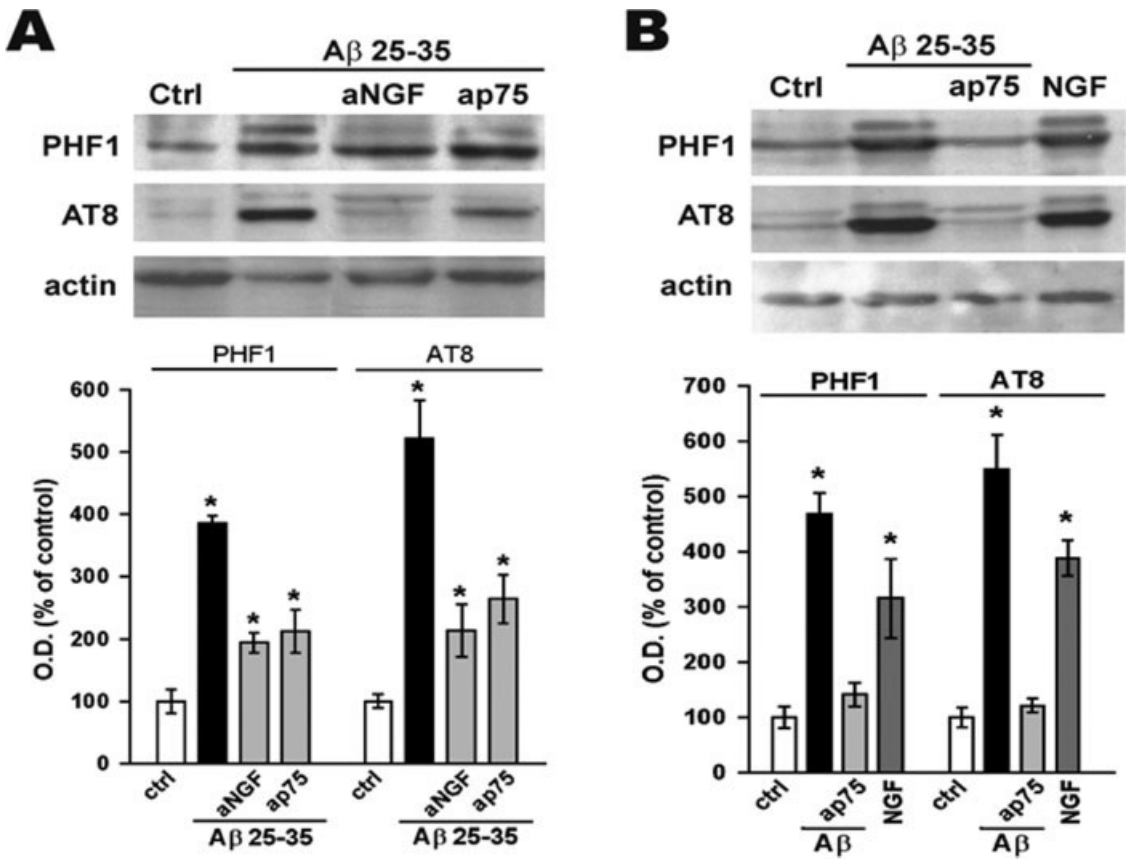

Fig. 2. Aβ-stimulated astrocytes induce p75-dependent tau hyperphosphorylation in cocultured hippocampal neurons. **A:** Hippocampal astrocytes were previously stimulated for 24 hr with Aβ 25–35 (10 μM) or vehicle (Ctrl), washed and mounted on the top of cultured hippocampal neurons, and cocultures were maintained for 24 hr in the presence or absence of blocking antibodies to NGF or $p75^{NTR}$ (aNGF, ap75). Neuronal lysates were processed for tau Western blot with Alzheimer-specific epitope antibodies PHF1 and AT8. Actin was used as an internal control. Data are express as percentage of control (mean ± SD). $\star P = 0.05$ vs. control. **B:** Pure hippocampal cultures were exposed to conditioned media from vehicle (ctrl)- or Aβ-treated astrocytes in the presence or absence of blocking antibodies to $p75^{NTR}$ (ap75). After 24 hr, tau phosphorylation was determined by Western blot. NGF represents the levels of tau phosphorylation in hippocampal neurons cultured above unstimulated astrocyte monolayers in the presence of exogenous NGF (100 ng/ml). Data are express as percentage of control (mean ± SD). $\star P = 0.05$ vs. control.

NGF stimulates cholinergic function, improves memory, and prevents cholinergic neuron degeneration in AD and animal models (Fischer et al., 1987; Tuszynski et al., 1990, 2005). This protective activity is likely to be mediated by TrkA signaling pathways on affected cholinergic neurons. However, our data suggest that NGF may serve to eliminate damaged hippocampal neurons in those regions exhibiting astrocytosis and neuroinflammatory changes linked to β-amyloid accumulation in AD. NGF is considered as a relevant mediator in tissue inflammation (Levi-Montalcini, et al., 1996), and its levels increase in neuropathologies characterized by prominent astrocytosis, such as AD (Crutcher et al., 1993; Scott et al., 1995; Fahnestock et al., 1996; Hock et al., 2000). NGF is induced in cultured astrocytes after stimulation with different inflammatory stimuli, including lipopolysacchaide and fibroblast growth factor (Yoshida and Gage, 1991; Galve-Roperh et al., 1997; Pehar et al., 2004; Cassina et al., 2005). Although reactive astrocytes are a potential source of NGF, the contribution of microglial cells to the increased NGF levels in AD cannot be excluded. Microglial cells up-regulate NGF following activation (Krenz and Weaver, 2000) and accumulate in degenerating brain as part of the neuroinflammatory process occurring in AD (McGeer and McGeer, 2003).

Hippocampal neuron death induced by astrocyte-derived NGF involved tau hyperphosphorylation. This effect was mediated by $p75^{NTR}$, since it was prevented by $p75^{NTR}$ blocking antibodies. In accordance, a large proportion of $p75^{NTR}$-expressing neurons in the CA1 and CA2 hippocampal subfields of AD patients present tau hyperphosphorylation (Hu et al., 2002). Abnormal tau phosphorylation results in dysfunctional axonal transport, leading to tangle formation, altered synaptic structures, and impaired mitochondrial transport, with subsequent energy depletion and the neuronal atrophy observed in AD (Rappaport, 2003; Johnson and Stooth-

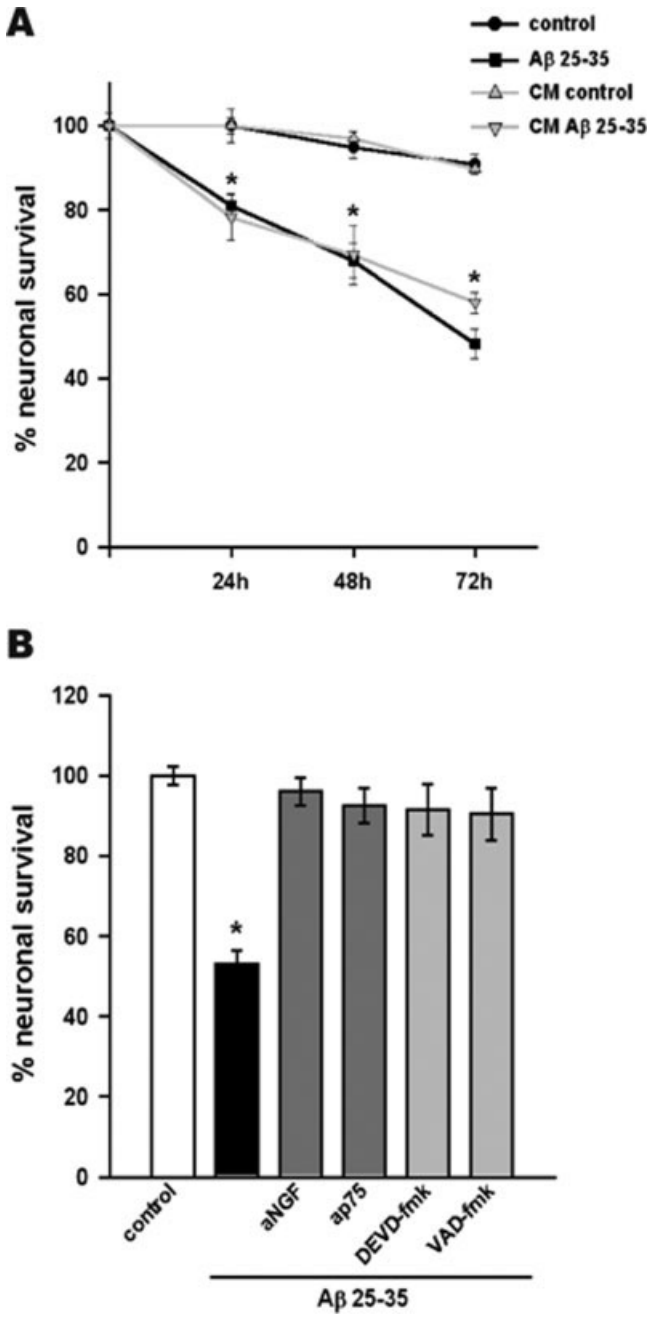

Fig. 3. Aβ-stimulated astrocytes induce p75-dependent neuronal apoptosis. **A:** Hippocampal astrocytes previously stimulated for 24 hr with vehicle (control) or 10 μM Aβ 25–35 were plated over hippocampal neuron cultures, and neuronal survival was determined at the indicated times. In a separate set of experiments, pure hippocampal neuron cultures were maintained in the presence of conditioned media collected from astrocytes cultures treated with vehicle (CM control) or Aβ (10 μM; CM Aβ 25–35). Neuronal survival was determined at the indicated times. Data are express as percentage of its respective control (mean ± SD). ⋆$P = 0.05$ vs. control. **B:** The cocultures established as described in A were maintained in the presence of NGF or $p75^{NTR}$ blocking antibodies (aNGF, ap75) or the caspase inhibitors DEVD-fmk (10 μM) and VAD-fmk (10 μM). Neuronal survival was determined after 72 hr. Data are expressed as percentage of control (mean ± SD). ⋆$P = 0.05$ vs. control.

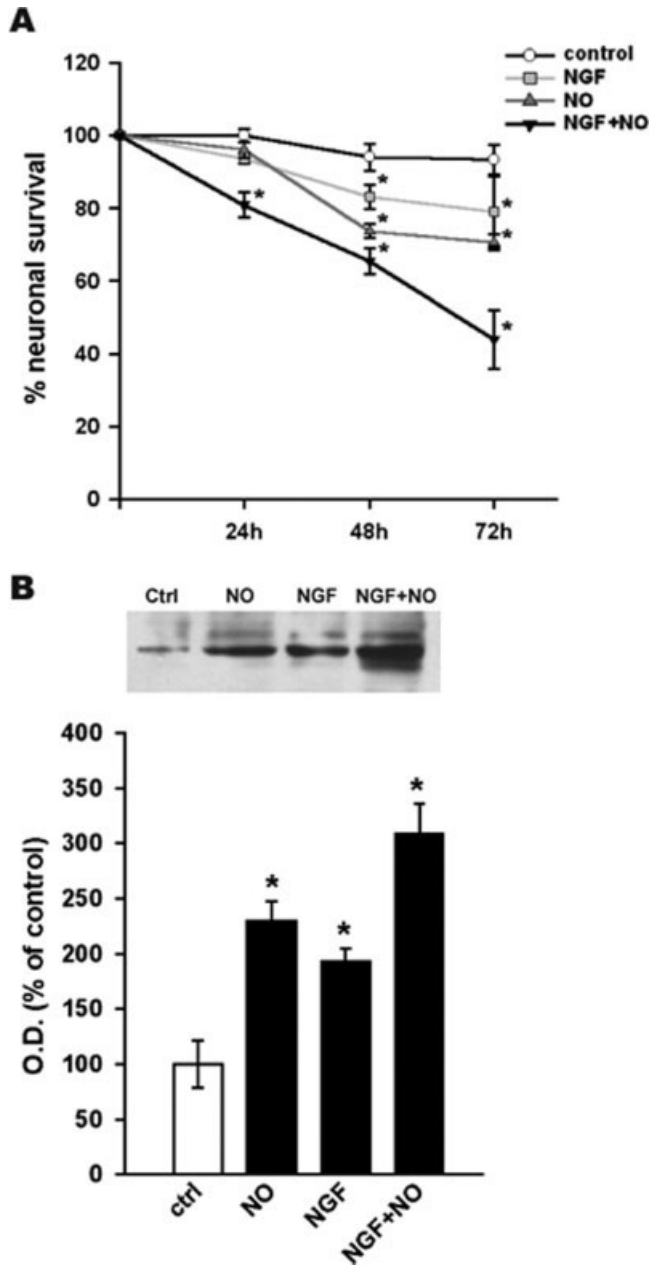

Fig. 4. Nitric oxide contributes to NGF-induced tau hyperphosphorylation and neuronal death. **A:** Pure hippocampal neuron cultures were treated with vehicle (control), NGF (100 ng/ml), NO (10 μM), or NGF plus NO, and neuronal survival was determined at the indicated times. Data are expressed as percentage of control (mean ± SD). **B:** Extracts from hippocampal neurons treated as indicated in A were assessed for tau phosphorylation by Western blot probed with PHF1 antibody. Data are express as percentage of control (mean ± SD). ⋆$P = 0.05$ vs. control.

off, 2004). In vivo, tau phosphorylation is regulated, directly or indirectly, by several kinases, including GSK3β, Cdk5, protein kinase A, and MARK (Maccioni et al., 2001; Alvarez et al., 2001; Johnson and Stoothoff, 2004). Further research is needed to determine whether

NGF signaling through $p75^{NTR}$ can directly activate these kinases, contributing to the formation of tangles in $p75^{NTR}$-expressing neurons in AD.

Oxidative or nitrative stress induced by NO production and peroxinitrite formation has been implicated in the AD pathogenesis (Smith et al., 1997; Hensley et al., 1998; Giasson et al., 2002; Nathan et al., 2005). We have previously shown that Aβ-stimulated astrocytes induced tau hyperphosphorylation in cocultured hippocampal neurons by a mechanism involving NO production (Sáez et al., 2004). Hippocampal and spinal cord astrocytes in culture produced sufficient NO to potentiate the deleterious effects of NGF (Sáez et al., 2004; Pehar et al., 2004). Aβ treatment stimulates NO production in hippocampal astrocytes by regulating both the inducible and the neuronal nitric oxide synthases (Sáez et al., 2004). Moreover, inhibition of astrocytic NO partially blocked tau hyperphosphorylation in cocultured hyppocampal neurons (Sáez et al., 2004). The partial prevention could be explained by the concomitant production of NGF by Aβ-stimulated astrocytes. The complete inhibition of neuronal hyperphosphorylation by $p75^{NTR}$ blocking antibodies was achieved only in neuronal cultures exposed to conditioned media from reactive astrocytes, effects mediated by NGF, insofar as NO is a short-life molecular species.

In the present study, we show that NO induced neuronal death and potentiated the effect of exogenous NGF in pure hippocampal cultures. Such an effect of NO may explain the increased tau hyperphosphorylation induced by NGF in cocultures compared with the effect on pure hippocampal neurons. Thus, our data suggest that hippocampal neurons expressing $p75^{NTR}$ may become vulnerable to NGF and NO secreted by surrounding activated astrocytes and that this mechanism may contribute to the progressive death of neurons in AD.

The role of $p75^{NTR}$ in the modulation of neuronal survival or death in AD is a matter of controversy. Several reports support the view that $p75^{NTR}$ expression protects neurons from degeneration, for example, through a downstream signaling involving nuclear factor-κB or the PI3K-Akt pathway (Culmsee et al., 2002; Bui et al., 2002). However, NGF and particularly its precursor form (pro-NGF), which is the predominant form of NGF in brain, is significantly increased in AD brain, and they induce neuronal apoptosis through $p75^{NTR}$ (Fahnestock et al., 2001; Pedraza et al., 2005). Moreover, the accumulation of pro-NGF in AD brain is correlated with loss of cognitive function (Peng et al., 2004). Our results support the view that NGF, or related species with specific apoptotic activity, is produced by Aβ-activated astrocytes and plays a role in mediating hippocampal neuron loss through $p75^{NTR}$ activation.

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