

Amyloid β Binds Trimers as Well as Monomers of the 75-kDa Neurotrophin Receptor and Activates Receptor Signaling*

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p75^{NTR}, a nerve growth factor co-receptor that has been implicated in apoptosis of neurons, is structurally related to Fas and the receptors for tumor necrosis factor- α that display ligand independent assembly into trimers. Using embryonic day 17 fetal rat cortical neurons and p75^{NTR}-expressing NIH-3T3 cells, we now show that p75^{NTR} exists as a trimer as well as a monomer. Furthermore, we have reported and others have confirmed that amyloid β binds p75^{NTR}, and that this binding leads to apoptotic cell death. We now report that amyloid β binds to trimers of p75^{NTR} as well as to p75^{NTR} monomers but not to the p140^{trkA}, the nerve growth factor co-receptor that mediates neuronal survival. Furthermore, amyloid β activates p75^{NTR}, strongly inducing the transcription of c-Jun mRNA and stimulating the stress-activated c-Jun NH₂-terminal kinase, as measured by phosphorylation of its substrate (glutathione S-transferase-c-Jun-(1–79)). Our data suggest that p75^{NTR} may be present as a preformed trimer that binds amyloid β to induce receptor activation, and support the hypothesis that p75^{NTR} activation by amyloid β is causally related to Alzheimer's disease.

The 75-kDa neurotrophin receptor (p75^{NTR}) is a transmembrane receptor that is a member of the TNF¹/Fas/CD40 superfamily of receptors (1). p75^{NTR} is one of the two transmembrane receptors for nerve growth factor (NGF) and other neurotrophins (2). In addition to p75^{NTR}, NGF binds a receptor of the trk family (p140^{trkA}) and p75^{NTR} can interact with p140^{trkA}, forming a complex (reviewed in Refs. 3 and 4) to modulate NGF actions, many of which involve signaling through p140^{trkA} (2). However, when p75^{NTR} is expressed in the absence of trk receptors or in the presence of low trk receptor levels, neurotrophin binding to p75^{NTR} alone can induce homodimer formation (5) and signal cellular apoptosis (2, 6, 7). It thus appears that activation of p75^{NTR} alone, like activation of other mem-

bers of this superfamily, may induce cellular apoptosis. We have reported (8) and others have confirmed (9) that amyloid- β (A β), a molecule implicated in the pathogenesis of Alzheimer's disease (AD) (10), specifically binds p75^{NTR} with an affinity close to that for neurotrophins and induces apoptosis of neurons and 3T3 cells engineered to constitutively express p75^{NTR}, but not the same cell line transfected with the plasmid vector alone. These data suggest that p75^{NTR}/A β interaction may mediate neuronal death in AD.

Indeed, much circumstantial evidence implicates p75^{NTR} in the pathogenesis of AD. AD initially affects the basal forebrain cholinergic neurons (11–13) that in the adult human brain express the highest levels of p75^{NTR} (14). Furthermore, cortical neurons of AD patients express p75^{NTR}, but this receptor is only infrequently expressed in cortical neurons of nonaffected aged-matched controls (15). Finally, the level of p140^{trkA}, the receptor that in coordination with p75^{NTR} appears to mediate the beneficial effects of NGF (2), is decreased in the human brain of AD patients (16–19), whereas p75^{NTR} levels are not (20, 21), suggesting that AD may result in part from a relative overexpression of p75^{NTR} that renders neurons more susceptible to A β -induced apoptosis (22).

The signaling pathways leading from p75^{NTR} ligand binding to apoptosis have been partially elucidated in various model systems. NGF binding to p75^{NTR} on the surface of cells lacking p140^{trkA} or expressing only low levels of p140^{trkA} may lead to ceramide generation through sphingomyelin hydrolysis (7). Ceramide generation is accompanied by activation of the Jun amino-terminal kinase (JNK) (7), allowing this family of kinases to phosphorylate and activate c-Jun and other transcription factors (23). The JNK pathway, and in particular strong JNK activation, are critical to apoptosis, as mice with knockout JNK genes show decreased neuronal apoptosis in response to the apoptotic inducing agent glutamate, as well as decreased apoptosis during development (23, 24). It is still not clear whether up-regulation of NF κ B through p75^{NTR} (25) occurs independently of the apoptotic pathway and suppresses cell death signals, as is the case with the TNF- α receptor (26), or whether NF κ B and JNK activation are part of the same signaling pathway leading to apoptosis, as suggested by Kuner *et al.* (9).

Other receptors of this superfamily, in particular TNF- α receptors and Fas, were recently found to be present both as preformed transmembrane trimeric complexes and as individual monomeric receptors (27, 28), with apoptotic signaling initiated only when a ligand binds the trimeric form of the receptor (27, 28). In this report we demonstrate that, similar to other members of this superfamily of receptors, p75^{NTR} exists on the cell surface as a trimer as well as a monomer. We show that A β binds both the trimeric as well as the monomeric form of

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¹ The abbreviations used are: TNF, tumor necrosis factor; DTSSP, 3,3-dithiobis(sulfosuccinimidylpropionate); JNK, c-Jun NH₂-terminal kinase; E17, embryonic day 17; NF κ B, nuclear factor κ B; DMEM, Dulbecco's modified Eagle's medium; NGF, nerve growth factor; AD, Alzheimer's disease; A β , amyloid β .

p75^{NTR}, but not p140^{trkA}; and that A β binding to p75^{NTR} leads to c-Jun mRNA up-regulation and JNK activation. We propose that binding of A β to p75^{NTR} trimers is a key pathomechanism of AD.

EXPERIMENTAL PROCEDURES

Materials—Anti-human and anti-rat p75^{NTR} antibodies were purchased from Roche Molecular Biochemicals. A β -(1–40) was purchased from Bachem (Torrance, CA), and NGF was purchased from ICN Pharmaceuticals. ¹²⁵I A β -(1–40) was purchased from Peninsula, and ¹²⁵I-NGF was purchased from NEN. JNK assay kit was purchased from Stratagene. c-Jun and glyceraldehyde-3-phosphate dehydrogenase cDNAs were purchased from ATCC (catalog nos. 63026 and 57090, respectively).

Amyloid- β Preparations—Lyophilized high performance liquid chromatograph-purified A β , containing the first 40 amino acids of the amyloid- β peptide (1–40), or an identically handled peptide containing the first 40 amino acids synthesized in reverse order (40–1) as a negative control (Bachem), were dissolved in double-distilled H₂O at a concentration of 5 mg/ml, aliquoted, and frozen at –20 °C. Before use, preparations were thawed once, diluted in DMEM, and were either added directly to medium (monomeric, soluble), or incubated at 37 °C for \geq 24 h before addition to medium (aggregated).

Cell Culture—p75^{NTR}, pCMV-, and p140^{trkA}-NIH 3T3 cells (kindly provided by Dr. M. V. Chao, New York University Medical Center, New York, NY) and E17 fetal rat cortical neurons were generated and maintained as described (5). Briefly, NIH 3T3 cells were maintained in DMEM supplemented with 10% fetal bovine serum in the presence of penicillin (45 ng/ml), streptomycin (68 ng/ml), and hygromycin B (17.5 ng/ml). For definitive experiments cells were provided with serum-free DMEM containing transferrin (5 μ g/ml) and insulin (5 μ g/ml). Neuronal cultures were established from the cerebral cortex of E17 fetal rats (long Evans; Charles River Laboratories, Portage, MI) as described (29), with minor modifications. In brief, the cerebral cortices were removed, resuspended in 0.025% trypsin in Hanks' buffered saline solution buffered with 10 mM Hepes, and incubated at 37 °C. After 10 min, serum-containing medium supplemented with 0.05% DNase was added, and the content was centrifuged and suspended in serum-containing medium supplemented with 0.02% DNase. Cells were then dissociated by gentle trituration, filtered through 73- and 35- μ m screens, and the dissociated cells were resuspended in serum-free neurobasal medium (Invitrogen). Cells were plated in T75 tissue culture flasks precoated overnight with 2 μ g/cm² poly-D-lysine in PBS (30,000–70,000, pH 7.5, Sigma). Cultures were used 2 days after plating.

Immunoprecipitation—Cells in suspension were preincubated at 37 °C for 30 min with ¹²⁵I-A β (25 nM), followed by 3,3-dithiobis(sulfosuccinimidylpropionate) (DTSSP) (2 mM) (Pierce) for 30 min at 4 °C. Equal amount of proteins were immunoprecipitated with anti-human p75^{NTR} (NIH 3T3 cells) or anti-rat p75^{NTR} (neurons) antibodies as described (8). Immunoprecipitates were separated by 8% PAGE and exposed to autoradiography.

Binding Assays—p75^{NTR}- and p140^{trkA}-NIH 3T3 cells were incubated in binding medium (DMEM, 10 mM Hepes, 0.1 mg/ml cytochrome c, 0.01% Tween 80, 1 mg/ml bovine serum albumin) with 0.5 nM ¹²⁵I-NGF and increasing concentrations of cold aggregated A β or with 10 pM ¹²⁵I-NGF and increasing concentrations of cold NGF for 4 h at 15 °C as described (8). At the end of the incubation period, cells were rinsed in PBS and lysed in 1 N NaOH, and lysates were subjected to γ counting.

Western Blot Analysis—p75^{NTR}-NIH 3T3 cells were lifted with EDTA and incubated at room temperature for 30 min with DTSSP (2 mM). Proteins were extracted in radioimmunoprecipitation assay buffer, and then half of the preparation was resuspended in Laemmli buffer containing 2-mercaptoethanol and the other half in buffer containing no reducing agents. Samples were processed for Western blotting and reacted with anti-human p75^{NTR} antibodies as described (30). The same number of cells were incubated in suspension at 37 °C with A β (20 μ M) or diluent for 30 min followed by DTSSP (2 mM) for 30 min at 4 °C. Cells were lysed and processed for Western blotting and reacted with anti-human p75^{NTR} (3T3 cells) or anti-rat p75^{NTR} (neurons) antibodies as described (30).

JNK Activation—p75^{NTR}- and pCMV-NIH 3T3 cells were supplemented with A β (20 μ M) or diluent for 30 min at 37 °C. Cells were then lysed in 25 mM HEPES, pH 7.7, 300 mM NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, pH 8.0, 0.1% Triton X-100, 0.5 mM dithiothreitol, 20 mM glycerophosphate, 0.1 mM Na₃VO₄, 2 μ g/ml leupeptin, and 100 μ g/ml phenylmethylsulfonyl fluoride. 50 μ g of lysate was used to determine JNK

activation using the JNK assay kit and following the instructions of the manufacturer.

Densitometric Analysis—Autoradiograms were scanned (Microtec Scan Maker II) into a computer (Massachusetts Engineering). Band intensity was determined after background subtraction using the densitometric program Sigma Gel (Jandel Scientific, Corte Madera, CA).

Statistical Analysis—JNK activation was analyzed by Univariate ANOVA and LSD *post hoc* analysis. c-Jun mRNA induction was analyzed by SPSS General Linear Model in repeated measure design. Both analyses used the SPSS Inc. statistical package version 9.0.

RESULTS

p75^{NTR} Is Assembled as a Trimer as Well as a Monomer—p75^{NTR} is structurally related to Fas and TNF- α receptors that self-assemble as trimers in the absence of ligand. To determine whether p75^{NTR} also exists in these two forms, rat cortical neurons (Fig. 1A) and p75^{NTR}-NIH 3T3 cells (Fig. 1B) were provided a reducible chemical cross linker and processed for Western blotting. Anti-p75^{NTR} specific antibodies revealed a 75-kDa band as well as a band at ~220–230 kDa. The same parental NIH 3T3 cell line transfected with the pCMV plasmid vector alone (pCMV-NIH 3T3 cells) was negative for p75^{NTR} immunoreactivity (Fig. 1C), confirming the specificity of the identified bands. As determined by densitometric analysis, the ratio between the 75-kDa band and the 220–230-kDa band (75 kDa/220–230 kDa) was 1.4 in the nonreduced samples (Fig. 1D). However, when samples were incubated with a reducing agent the ratio between the bands increased to 9.9, consistent with the presence of p75^{NTR} trimers as well as monomers in the cell membrane in the absence of ligand. Still, our experiments do not completely exclude the possibility that the 220–230-kDa band may represent p75^{NTR} trimerized by an autocrine/paracrine neurotrophic ligand, and/or that the high molecular mass band represents a complex containing another protein not detected by anti-p75^{NTR} antibodies.

A β Binds p75^{NTR} Trimer as Well as p75^{NTR} Monomer—When A β is incubated in aqueous solution for 5 days, as in the present experiments, the A β that is present in a soluble state migrates at an electrophoretic mobility of ~4.5 kDa, whereas aggregated A β is either retained at the top of the gel or migrates at a lower electrophoretic mobility of ~14 kDa (8, 41). To determine whether A β binds p75^{NTR} trimers, cells were stimulated with aggregated ¹²⁵I-A β , a preparation that contains a mixture of soluble and aggregated A β , in the presence of a chemical cross-linker and immunoprecipitation was performed with antibodies specific for p75^{NTR}. Autoradiography initially revealed a band of 230–240 kDa (Fig. 2A), consistent with a complex containing three 75-kDa receptors and an A β aggregate, but not excluding a complex containing three 75-kDa receptors and one or more soluble nonaggregated A β peptides. Longer exposure (24 h) revealed in addition a band of ~80 kDa (Fig. 2B), consistent with a complex containing one 75-kDa receptor and one 4.5-kDa A β peptide, but not strictly excluding a complex containing one 75-kDa receptor and an A β aggregate (~14 kDa). These data are consistent with A β binding to both forms of the receptor.

A β Does Not Bind p140^{trkA}—Because A β , particularly the aggregated form, is known to be “sticky,” to rule out the possibility that the above results represent nonspecific binding, p75^{NTR}-NIH 3T3 cells were incubated with 0.5 nM ¹²⁵I-NGF and increasing concentrations of unlabeled aggregated A β . A β competitively displaced ¹²⁵I-NGF binding in p75^{NTR}-NIH 3T3 cells (Fig. 3A). Regression analysis showed significant dose-dependent inhibition of binding with increasing A β concentrations ($r^2 = 0.94$, $p < 0.006$; Jandel Scientific). Because neurons express two NGF receptors, to rule out the possibility that A β also binds the nerve growth factor co-receptor p140^{trkA}, p140^{trkA}-NIH 3T3 cells were incubated with ¹²⁵I-NGF and A β as above.

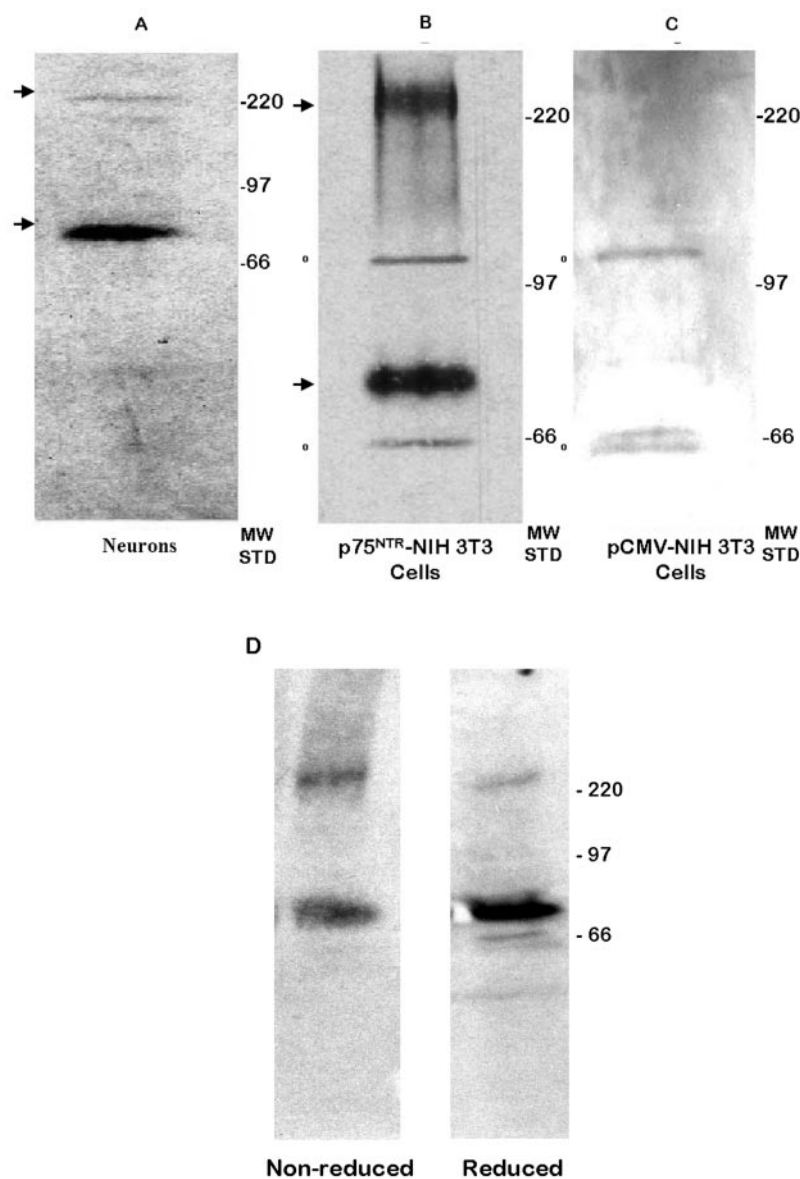


FIG. 1. $p75^{NTR}$ is assembled as a trimer as well as a monomer. E17 fetal rat cortical neurons (A), $p75^{NTR}$ -NIH 3T3 cells (B and D), and pCMV-NIH 3T3 cells (C) were provided DTSSP and then were processed for Western blot analysis using anti-rat (A) or anti-human (B and C) $p75^{NTR}$ antibodies. The antibodies identified a 75-kDa band as well as a band of ~220–230 kDa molecular mass only in rat cortical neurons (A) and $p75^{NTR}$ -NIH 3T3 cells (B). The ratio between the 75-kDa band and the 220–230-kDa band (75 kDa/220–230 kDa) was 1.4 in the non-reduced samples and 9.9 in the reduced samples (D), consistent with the presence of $p75^{NTR}$ trimers as well as monomers, but not excluding the possibility of a monomeric or dimeric form of $p75^{NTR}$ and as yet unidentified additional protein with a very similar molecular weight. Nonspecific bands (B and C) are marked by open circles.

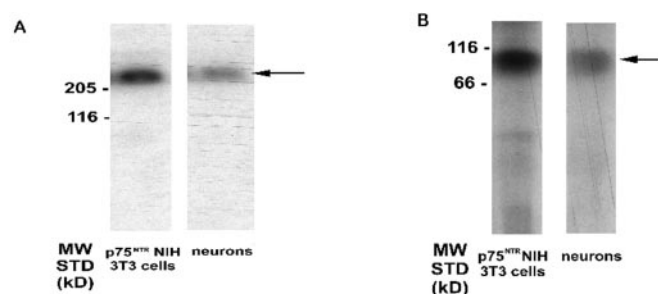


FIG. 2. Aggregated $A\beta$ binds $p75^{NTR}$ trimer as well as $p75^{NTR}$ monomer. $p75^{NTR}$ -NIH 3T3 cells and E17 fetal rat cortical neurons were stimulated with aggregated ^{125}I - $A\beta$ as described under "Experimental Procedures." Immunoprecipitation with anti- $p75^{NTR}$ antibodies followed by autoradiography showed initially a band of 230–240 kDa (A) and with longer exposure an additional band of 80–90 kDa (B).

$A\beta$ did not affect ^{125}I -NGF binding to $p140^{trkA}$ -NIH 3T3 (Fig. 3B), whereas nonlabeled NGF competitively displaced ^{125}I -NGF binding in the cells (Fig. 3C), further supporting the specificity of $A\beta$ binding to $p75^{NTR}$.

$A\beta$ Binding to $p75^{NTR}$ Induces *c-Jun* mRNA—To determine whether $A\beta$ signals through $p75^{NTR}$, $p75^{NTR}$ -NIH 3T3 cells, pCMV-NIH 3T3 cells, and rat cortical neurons were stimulated

with aggregated $A\beta$ and the induction of *c-Jun* mRNA was determined. Northern blot analysis showed rapid *c-Jun* mRNA up-regulation in $p75^{NTR}$ -NIH 3T3 cells (Fig. 4A) and in neurons (Fig. 4C) but not in control cells transfected with the plasmid vector alone (Fig. 4A). In a total of four independent experiments, *c-Jun* mRNA was significantly induced by $A\beta$ ($p < 0.05$) in both $p75^{NTR}$ -expressing cell types but not in pCMV-NIH 3T3 cells that do not express the receptor. This result is consistent with *c-Jun*-mediated signal transduction by $A\beta$ through $p75^{NTR}$.

$A\beta$ Binding to $p75^{NTR}$ Activates JNK— $p75^{NTR}$ -NIH 3T3 cells and pCMV-NIH 3T3 were stimulated with aggregated $A\beta$, and JNK activation was measured by phosphorylation of its substrate GST-*c-Jun*-(1–79). Strong JNK activation was detected in $p75^{NTR}$ -NIH 3T3 cells, but JNK was only minimally activated in control pCMV-NIH 3T3 cells that were handled identically (Fig. 5, A and B). In a total of four independent analyses, JNK activation was significantly induced by $A\beta$ only in cells that express $p75^{NTR}$ ($p < 0.02$). There was no statistically significant difference between $A\beta$ -stimulated pCMV-NIH 3T3 cells that do not express $p75^{NTR}$ versus the same cells stimulated with diluent, or between $p75^{NTR}$ -NIH 3T3 cells stimulated with diluent versus pCMV-NIH 3T3 cells. This result is

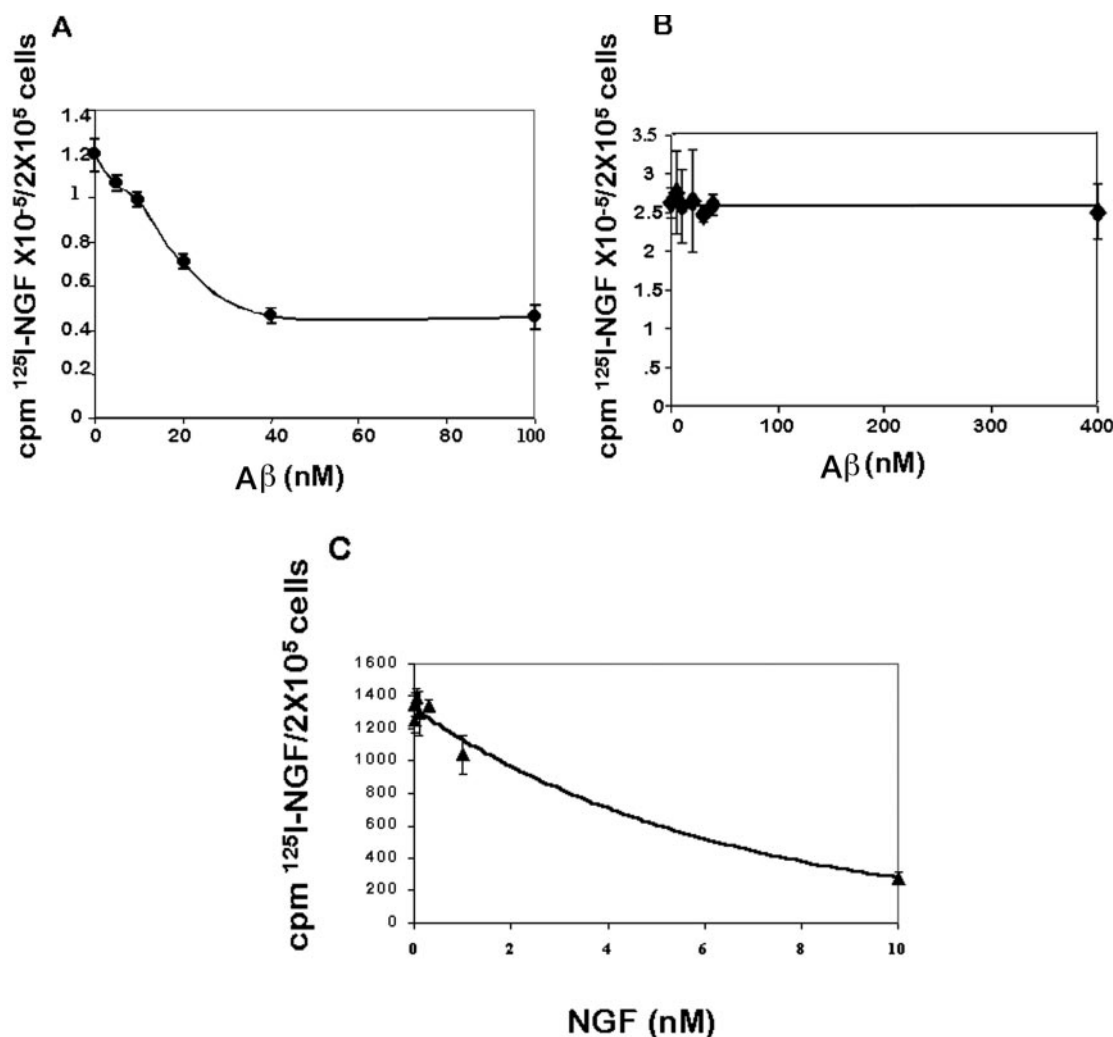


FIG. 3. **A β does not bind p140^{trkA}.** p75^{NTR}-NIH 3T3 cells and p140^{trkA}-NIH 3T3 cells were incubated for 4 h in binding medium with 0.5 nM ¹²⁵I-NGF and increasing concentrations of unlabeled aggregated A β (0–400 nM). A concentration-dependent inhibition of ¹²⁵I-NGF binding by A β to p75^{NTR}-NIH 3T3 cells was observed (A). A β did not affect ¹²⁵I-NGF binding to p140^{trkA}-NIH 3T3 cells (B), but NGF inhibited ¹²⁵I-NGF binding to these cells in a concentration-dependent manner (C). Each point is the average of at least two separate determinations.

consistent with previous reports that apoptosis induced by NGF in oligodendrocytes that express only p75^{NTR} is mediated by JNK activation (7).

DISCUSSION

p75^{NTR} belongs to the family of cell surface “death receptors” that include Fas and the TNF- α receptors. It was initially thought that these receptors trimerize only upon ligand binding (31). However, it was recently shown that both TNF- α receptors and Fas can be found in a complex containing three monomeric receptors in the absence of exogenously added ligand, with further analysis confirming that an extracellular domain on the receptor and not an external ligand is responsible for the trimerization (confirming that at least a portion of these receptors are present as preformed trimers) (27, 28). Using p75^{NTR} NIH-3T3 cells and rat cortical neurons, we employed a chemical that cross-links only molecules that are less than 12 Å apart. We identified two forms of p75^{NTR}, a monomeric form of 75 kDa and a form of ~220–230 kDa, consistent with a p75^{NTR} trimer, suggesting that a subset of p75^{NTR} are present as trimers on the cell surface. However, we cannot completely exclude the possibility that the higher molecular mass band is a complex that contains one or more other proteins, in addition to p75^{NTR}, that happen to be within a 12-Å distance of p75^{NTR} and whose aggregate molecular mass is 75

or 150 kDa, resulting in the observed band of ~220–230 kDa. Still, this seems unlikely because the band was present in two unrelated cell types, primary cortical neurons and p75^{NTR} NIH-3T3 cells, and no other specific bands of different molecular mass were recognized by the antibodies. Furthermore, disruption of the cross-linking led to increased intensity of the 75-kDa band and decreased intensity of the 220–230-kDa band, with a redistribution of p75^{NTR} immunoreactivity quantitatively most consistent with disaggregation of trimers into monomers. Neither can we completely exclude the possibility that the higher molecular mass band represents receptor trimerization as a result of autocrine neurotrophic factors produced by the 3T3 cells (30, 32) or paracrine neurotrophic factors produced by contaminating cells, other than neurons, in the fetal rat brain cultures (reviewed in Refs. 33 and 34).

Although TNF- α receptors are present as preformed trimers, these receptor complexes do not signal in the absence of ligand binding (28). Interestingly, preventing TNF- α receptor ligand-independent self-association eliminated TNF- α binding, suggesting that homotrimeric TNF- α can only bind the pre-assembled trimeric receptor (28). To determine whether A β binds p75^{NTR} trimers as the first step in the previously reported induction of apoptosis (8, 9), aggregated ¹²⁵I-A β , a mixture of both aggregated and soluble peptides, was added to neurons

FIG. 4. $\text{A}\beta$ binding to $p75^{\text{NTR}}$ induces c-Jun RNA. $p75^{\text{NTR}}$ -NIH 3T3 cells, pCMV-NIH 3T3 cells (A and B), or rat cortical neurons (C and D) were stimulated with aggregated $\text{A}\beta$. Total cellular RNA was harvested at different intervals after stimulation and processed for Northern blotting. The 2.7- and 3.2-kb c-Jun transcripts were strongly induced only in $p75^{\text{NTR}}$ -NIH 3T3 cells and rat cortical neurons but not in pCMV NIH 3T3 cells. The lower panels of A and C are loading controls (18 S, 28 S, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA, respectively). B and D, graphic representation of band intensity as determined by densitometric analysis of the autoradiograms.

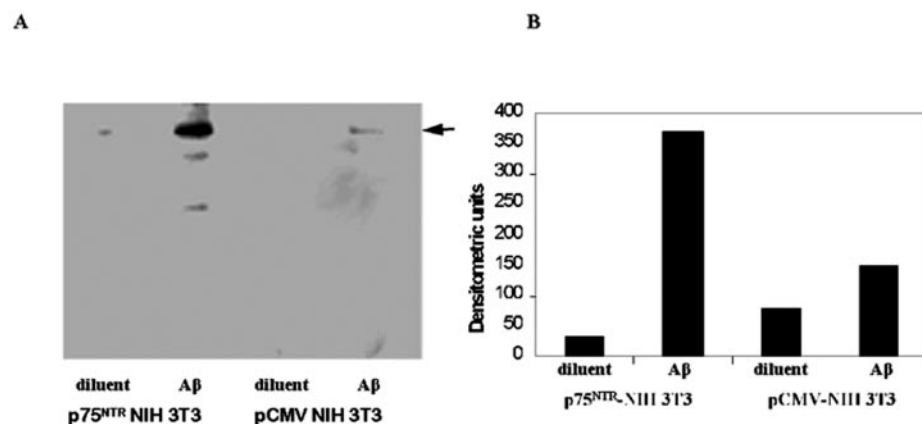
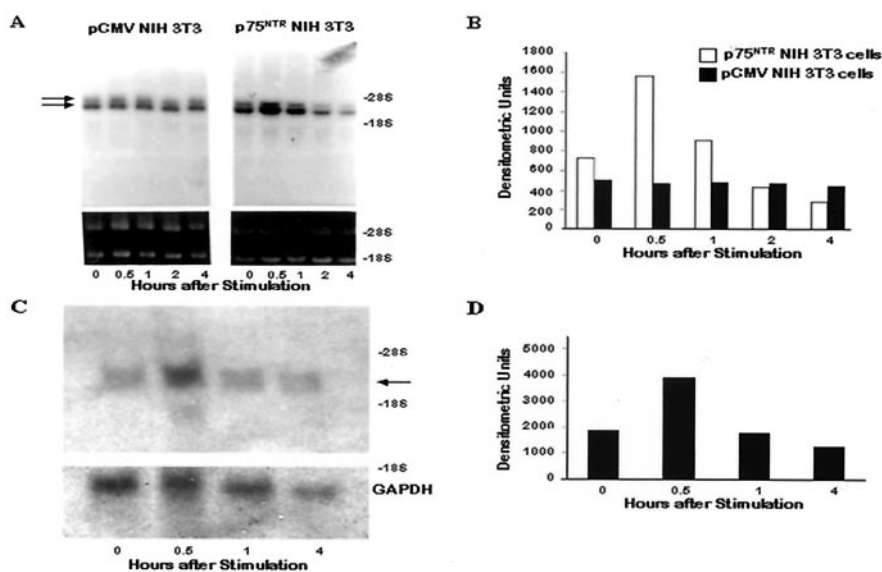


FIG. 5. $\text{A}\beta$ binding to $p75^{\text{NTR}}$ activates JNK. A, $p75^{\text{NTR}}$ -NIH 3T3 cells and pCMV-NIH 3T3 cells were stimulated with aggregated $\text{A}\beta$ or diluent and JNK activation was determined by phosphorylation of its substrate (GST-c-Jun-(1-79)). Autoradiography demonstrates a strong band representing substrate phosphorylation in $p75^{\text{NTR}}$ NIH 3T3 cells stimulated with $\text{A}\beta$. B, graphic representation of band intensity as determined by densitometric analysis of the autoradiogram.

and to $p75^{\text{NTR}}$ -NIH 3T3 cells. Immunoprecipitation experiments showed that $\text{A}\beta$ bound both the monomeric and the trimeric forms of the receptor. We speculate that because only aggregated $\text{A}\beta$ induces cellular apoptosis (11), only the aggregated form of $\text{A}\beta$ activates pre-assembled $p75^{\text{NTR}}$; and that this induces neuronal apoptosis. However, the result of this experiment does not allow us to rule out the possibility that it is the soluble form of $\text{A}\beta$ that binds $p75^{\text{NTR}}$ trimer.

We also documented that addition of cold $\text{A}\beta$ inhibited ^{125}I NGF binding to $p75^{\text{NTR}}$ in cells expressing the receptor but not in the same cells engineered to express $p140^{\text{trkA}}$, the other recognized receptor for NGF. This result and the previously reported high affinity of $\text{A}\beta$ for $p75^{\text{NTR}}$ (8) strongly support our conclusion that $\text{A}\beta$ binding to $p75^{\text{NTR}}$ is specific and is not the result of the aggregated molecule being nonspecifically sticky.

Our studies also demonstrate that $\text{A}\beta$ binding to $p75^{\text{NTR}}$ induces receptor signaling events. In both $p75^{\text{NTR}}$ NIH 3T3 cells and cortical neurons, there was a rapid and transient induction of c-Jun transcripts following stimulation with $\text{A}\beta$. In addition, there was strong activation of JNK. This result strongly suggests that c-Jun induction is a specific signaling event that follows $\text{A}\beta$ binding to $p75^{\text{NTR}}$. It cannot be explained by $\text{A}\beta$ activation of another receptor or by $\text{A}\beta$ activating a non-receptor signaling pathway because these events were not observed in the control cell line engineered to express the plasmid vector alone, identical to the responsive cells but for the expression of $p75^{\text{NTR}}$. Indeed, c-Jun is one of the immediate early genes that are induced in response to a variety of stimuli including apoptotic stimuli (35, 36). Furthermore, other data

suggest that c-Jun/AP-1 is essential for the regulation of apoptosis (35), making induction of the c-Jun/AP-1 complex in the present experiments consistent with $\text{A}\beta$ exposure mediating apoptosis of cells expressing $p75^{\text{NTR}}$ (8).

Some investigators have reported that apoptosis is associated with sustained increase in c-Jun mRNA levels (37, 38). However, sustained c-Jun induction has not been a consistent finding preceding cellular apoptosis, and several investigators (35, 36, 39) have shown that rapid and transient c-Jun induction, very similar to that observed by us, induces apoptosis. Thus, sustained c-Jun induction does not appear to be required for apoptosis and may instead reflect a cell-specific or stimulus-specific response.

JNK comprises a family of enzymes that phosphorylate c-Jun and are strongly activated in cells exposed to apoptotic stimuli (40). We observed a strong activation of JNK within 30 min after $\text{A}\beta$ supplementation in cells expressing $p75^{\text{NTR}}$ but not in cells lacking the receptor. In combination with our previous demonstration of apoptosis in these cells under the same conditions (8), this indicates that $\text{A}\beta$ induced apoptosis is mediated through the AP-1 complex. The data in combination show that $\text{A}\beta$ binds and activates $p75^{\text{NTR}}$, inducing c-Jun mRNA and presumably c-Jun protein with subsequent activation of JNK and c-Jun phosphorylation. The signaling pathway almost certainly then interacts with others to produce apoptosis, although our studies do not address this point.

In summary, we have shown that $p75^{\text{NTR}}$, like other death receptors, is present as trimers as well as monomers on the surface of neurons; and that $\text{A}\beta$ binds to $p75^{\text{NTR}}$ trimers as well as to $p75^{\text{NTR}}$

monomers but not to p140^{trkA}. We have further shown that A β binding induces receptor activation and propagates intracellular signaling that leads to apoptosis. The data strongly imply that A β /p75^{NTR} interaction is causally related to neuronal loss in AD. Therapeutic interventions that block A β /p75^{NTR} interaction might thus slow or arrest the progression of AD.

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