BETA AMYLOID BINDS TRIMERS AS WELL AS MONOMERS OF THE 75 kD NEUROTROPHIN RECEPTOR AND ACTIVATES RECEPTOR SIGNALING

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Running title: Aβ signaling through $p75^{NTR}$

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Summary

$p75^{NTR}$, 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 E17 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, independent of ligand binding. 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 NH_2-terminal kinase (JNK) as measured by phosphorylation of its substrate [GST-cJun (1-79)]. Our data suggest that $p75^{NTR}$ is present as a preformed complex 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.

Introduction

The 75 kD neurotrophin receptor ($p75^{NTR}$) is a transmembrane receptor that is a member of the TNF/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 $p140^{trkA}$ 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 signal cellular apoptosis (2-4). It thus appears that activation of $p75^{NTR}$ alone, like activation of other members of this superfamily, would induce cellular apoptosis. Recently, we reported (5) and others confirmed (6) that amyloid-β (Aβ), a molecule implicated in the pathogenesis of Alzheimer's Disease (AD) (7), 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 $p75^{NTR}$/Aβ 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 (8-10) that in the adult human brain express the highest levels of $p75^{NTR}$ (11). Furthermore, cortical neurons of AD patients express $p75^{NTR}$, but this receptor is only infrequently expressed in cortical neurons of non-affected aged-matched controls (12). 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 (13-

16), while $p75^{NTR}$ levels are not (17,18), suggesting that AD may result in part, from a relative over-expression of $p75^{NTR}$ that renders neurons more susceptible to Aβ induced apoptosis (19).

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 (4). Ceramide generation is accompanied by activation of the Jun amino-terminal kinase (JNK) (4), allowing this family of kinases to phosphorylate and activate c-Jun and other transcription factors (20). 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 (20,21). It is still not clear whether upregulation of NFKB through $p75^{NTR}$ (24) occurs independently of the apoptotic pathway and suppresses cell death signals, as is the case with the TNF-α receptor (23), or whether NFKB and JNK activation are part of the same signaling pathway leading to apoptosis as suggested by Kuner et al (6).

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 (24,25), with apoptotic signaling initiated only when a ligand binds the trimeric form of the receptor (24,25). In this manuscript we demonstrate that, similar to other members of this superfamily of receptors, $p75^{NTR}$ exists on the cell surface as a preformed trimer as well as a monomer. We show that Aβ binds both the trimeric as well as the monomeric form of $p75^{NTR}$,

but not $p140^{trkA}$; and that Aβ binding to $p75^{NTR}$ leads to c-jun mRNA up-regulation and JNK activation. We propose that binding of Aβ 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. Aβ 1-40, Aβ 40-1 were purchased from Bachem. ^{125}I Aβ 1-40 was purchased from Peninsula and ^{125}I-NGF was purchased from NEN. JNK assay kit was purchased from Stratagene. c-jun and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNAs were purchased from ATCC (#63026 and #57090, respectively).

β-Amyloid preparations

Lyophilized HPLC-purified Aβ, containing the first 40 amino acids of the β-amyloid 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 California, Torrance, CA), were dissolved in ddH_2O 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$24h before addition to medium (aggregated).

Cell culture

$p75^{NTR}$- and pCMV- NIH 3T3 cells (kindly provided by Dr. M.V.Chao, New York University Medical Center, New York) and E17 fetal rat cortical neurons were generated and maintained as described (5). Briefly, NIH 3T3 cells were maintained in DMEM supplemented with 10% FBS 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 μg/ml) and insulin (5 μg/ml). Neuronal cultures were established from the cerebral cortex of E17 fetal rats (long Evans; Charles River Lab, Portage, MI) as described (26), with

minor modifications. In brief, the cerebral cortices were removed, re-suspended in 0.025% trypsin in HBSS 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 73.7-μm and 35-μm screens, and the dissociated cells were resuspended in serum-free neurobasal medium (GIBCO BRL). Cells were plated in T75 tissue culture flasks precoated overnight with 2 $\mu g/cm^2$ poly D-lysine in PBS (30,000-70,000, pH 7.5, Sigma Chemical Co.). Cultures were used 2 d after plating.

Immunoprecipitation

Cells in suspension were preincubated at 37°C for 30 min with ^{125}I-Aβ (25 nM) followed by 3,3-dithiobis [sulfasuccinimidyl proprionate] (DTSSP) (2 mM) (Pierce, Rockford, IL) 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 (5). 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, 10mM Hepes, 0.1 mg/ml cytochrome C, 0.01% Tween 80, 1mg/ml BSA) with 0.5 nM ^{125}I NGF and increasing concentrations of cold aggregated Aβ for 4h at 15^0C as described (5). At the end of the incubation period, cells were rinsed in PBS, 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 minutes with DTSSP (2mM). Proteins were extracted in RIPA buffer and then half 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 (27). The same number of cells were incubated in suspension at 37°C with Aβ (20 uM) 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 (27).

JNK activation

$p75^{NTR}$ NIH 3T3 cells were preincubated with the cyclic peptide or diluent in DME supplemented with 0.1% FBS for 24 hours and then supplemented with Aβ (20 uM) for 30 min at 37°C. Cells were then lysed in 25 mM HEPES, pH 7.7, 300 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA, pH 8.0, 0.1% Triton X-100, 0.5 mM DTT, 20 mM glycerophosphate, 0.1 mM Na_3VO_4, 2 μg/ml leupeptin and 100 μg/ml PMSF. 50 ug 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 (Microtek 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 UNIANOVA with Tukey-HSD 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 if $p75^{NTR}$ 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 kD band as well as a band at approximately 225-230 kD molecular weight. 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 75kD band and the 220-230 kD band (75 kD/220-230kD) was 1.4 in the non reduced samples. 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.

Aggregated Aβ binds $p75^{NTR}$ trimer as well as $p75^{NTR}$ monomer

When Aβ is incubated in aqueous solution for 5 days, as in the present experiments, the Aβ that is present in a soluble state migrates at an electrophoretic mobility of ~4.5 kD while aggregated Aβ is either retained at the top of the gel or migrates at a lower electrophoretic mobility of ~14 kD (5,28). To determine if Aβ binds $p75^{NTR}$ trimers, cells were stimulated with aggregated ^{125}I-Aβ 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 kD (Fig 2A), consistent with a complex containing three 75 kD receptors and an Aβ aggregate, but not

excluding a complex containing three 75 kD receptors and a soluble non-aggregated Aβ peptide. Longer exposure (24 hours) revealed in addition a band of approximately 80 kD (Fig. 2B), consistent with a complex containing one 75 kD receptor and one 4.5 kD Aβ peptide, but not excluding a complex containing one 75 kD receptor and Aβ aggregate (~14 kD).

To better define the molecular weight of the observed bands we repeated the experiment using cold aggregated Aβ or soluble Aβ in the presence of DTSSP and identified $p75^{NTR}$ by Western blot analysis. In both rat cortical neurons (Figure 2C) and $p75^{NTR}$-NIH 3T3 cells (Figure2D), anti $p75^{NTR}$ antibodies revealed the formation of an ~80 kD band in addition to a 230-240 kD band. The lower band appeared more consistent with $p75^{NTR}$ monomer bound to Aβ monomer, as aggregated Aβ bound to $p75^{NTR}$ monomer would be expected to display a band of ~90 kD. Also, in cells stimulated with aggregated Aβ, the higher molecular weight band was consistently stronger than the comparable band in cultures stimulated with soluble Aβ, suggesting that aggregated Aβ promotes receptor trimerization. These data are consistent with the presence of $p75^{NTR}$ trimers as well as monomers in the cell membrane prior to addition of Aβ, and with Aβ binding to both forms of the receptor.

Aggregated Aβ does not bind $p140^{trkA}$

Because aggregated Aβ is known to be "sticky," to rule out the possibility that the above results represent non-specific binding, $p75^{NTR}$-NIH 3T3 cells were incubated with 0.5 nM ^{125}I-NGF and increasing concentrations of unlabeled aggregated Aβ. Aβ competitively displaced ^{125}I-NGF binding in $p75^{NTR}$-NIH 3T3 cells (Fig 3A). Regression analysis showed significant dose dependent inhibition of binding with increasing Aβ concentrations (r^2=0.94, $p<0.006$; Jandel Scientific). Because neurons express two NGF receptors, to rule out the possibility that Aβ also

binds the nerve growth factor co-receptor –$p140^{trkA}$, $p140^{trkA}$-NIH 3T3 cells were incubated with ^{125}I-NGF and Aβ as above. Aβ did not affect ^{125}I-NGF binding to $p140^{trkA}$ –NIH 3T3 (Fig 3B), further supporting the specificity of Aβ binding to $p75^{NTR}$.

Aggregated Aβ binding to $p75^{NTR}$ induces c-jun mRNA

To determine if Aβ signals through $p75^{NTR}$, $p75^{NTR}$-NIH 3T3 cells, pCMV-NIH 3T3 cells and rat cortical neurons were stimulated with aggregated Aβ and the induction of c-jun mRNA was determined. Northern blot analysis showed rapid c-jun mRNA upregulation in $p75^{NTR}$-NIH 3T3 cells (Fig 4A) and in neurons (Fig 4B) 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 Aβ ($p< 0.05$) 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 Aβ through $p75^{NTR}$.

Aggregated Aβ binding to $p75^{NTR}$ activates JNK

$p75^{NTR}$ -NIH 3T3 cells and pCMV-NIH 3T3 were stimulated with aggregated Aβ 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 5A&B). In a total of four independent analyses, JNK activation was significantly induced by Aβ only in cells that express $p75^{NTR}$ ($p< 0.02$). There was no statistically significant difference between Aβ stimulated pCMV-NIH 3T3 cells that do not express $p75^{NTR}$ vs. the same cells stimulated with diluent, or between $p75^{NTR}$-NIH 3T3 cells stimulated with diluent vs. pCMV-NIH 3T3 cells. This result is

consistent with previous reports that apoptosis induced by NGF in oligodendrocytes that express only $p75^{NTR}$ is mediated by JNK activation (4).

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 (29). 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 a ligand, confirming that at least a portion of these receptors are present as preformed trimers (24,25). Using $p75^{NTR}$ NIH-3T3 cells and rat cortical neurons, we employed a chemical that cross links only molecules that are less than 12Å apart. In the absence of neurotrophins, we identified two forms of $p75^{NTR}$, a monomeric form of 75 kD and a form of approximately 220-230 kD, consistent with a $p75^{NTR}$ trimer, suggesting that a subset of $p75^{NTR}$ are present as trimers on the cell surface. However, we can not completely rule out the possibility that the higher molecular weight band is a complex that contains one or more other proteins, different from $p75^{NTR}$, that happen to be within a 12Å distance of $p75^{NTR}$ and whose aggregate molecular weight is 75 or 150 kD, resulting in the observed band of approximately 220-230 kD. 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 weight were recognized by the antibodies. Furthermore, disruption of the cross-linking lead to increased intensity of the 75kD band and decreased intensity of the 225-230kD band, strongly suggesting that the higher molecular weight band is indeed $p75^{NTR}$ trimer.

Although TNF-α receptors are present as preformed trimers, these receptor complexes do not signal in the absence of ligand binding (25). 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 (25). To determine if Aβ binds $p75^{NTR}$ trimers as the first step in the previously reported induction of apoptosis (5,6), aggregated ^{125}I-Aβ was added to neurons and to $p75^{NTR}$-NIH 3T3 cells. Immunoprecipitation experiments showed that Aβ bound both the monomeric and the trimeric forms of the receptor. We speculate that similar to TNF-α, aggregated Aβ bind (8) and subsequently activate (8) pre-assembled $p75^{NTR}$; and that this induces neuronal apoptosis.

We also documented that addition of cold Aβ inhibited ^{125}I NGF binding to $p75^{NTR}$ in cells expressing the receptor but not in the same cells engineered to express $p140^{trkA}$, the other recognized receptor for NGF. This result and the previously reported high affinity of Aβ for $p75^{NTR}$ (5) strongly support our conclusion that Aβ binding to $p75^{NTR}$ is specific and is not the result of the aggregated molecule being non-specifically "sticky".

Our studies also demonstrate that Aβ binding to $p75^{NTR}$ induce receptor signaling events. In both $p75^{NTR}$ NIH 3T3 cells and cortical neurons, there was a rapid and transient induction of c-jun transcripts following stimulation with Aβ. In addition, there was strong activation of JNK. This result strongly suggests that c-jun induction is a specific signaling event that follows Aβ binding to $p75^{NTR}$. It can not be explained by Aβ activation of another receptor or by Aβ 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 $p75^{NTR}$. Indeed, c-jun is one of the immediate early genes that is induced in response to a variety of stimuli including apoptotic stimuli (30). Furthermore, other data suggest that c-jun/AP-1 is essential for the regulation of apoptosis (31), making induction of the c-Jun/AP-1 complex in the present experiments consistent with Aβ exposure mediating apoptosis of cells expressing $p75^{NTR}$ (5).

Some investigators have reported that apoptosis is associated with sustained increase in c-jun mRNA levels (32,33). However, sustained c-jun induction has not been a consistent finding preceding cellular apoptosis, and several investigators (30,31,34) 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 (35). We observed a strong activation of JNK within 30 minutes after Aβ supplementation in cells expressing $p75^{NTR}$ but not in cells lacking the receptor. In combination with our previous demonstration of apoptosis in these cells under the same conditions (5), this indicates that Aβ induced apoptosis is mediated through the AP-1 complex. The data in combination show that Aβ binds and activates $p75^{NTR}$, inducing c-jun mRNA and presumptively 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 $p75^{NTR}$, like other "death receptors," is present as trimers as well as monomers on the surface of neurons; and that Aβ binds to $p75^{NTR}$ trimers as well as to $p75^{NTR}$ monomers but not to $p140^{trkA}$. We have further shown that Aβ binding induces receptor activation and propagates intracellular signaling that leads to apoptosis. The data strongly imply that Aβ/$p75^{NTR}$ interaction is causally related to neuronal loss in AD. Therapeutic interventions that block Aβ/$p75^{NTR}$ interaction might thus slow or arrest the progression of AD.

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Figure Legends

Figure 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, 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,C) $p75^{NTR}$ antibodies. The antibodies identified a 75 kD band as well as a band of ~225-230 kD molecular weight only in rat cortical neurons (A) and $p75^{NTR}$-NIH 3T3 cells (B). The ration between the 75kD band and the 220-230kD band (75kD/220-230kD) was 1.4 in the non-reduced samples and 9.9 in the reduced samples, consistent with the presence of $p75^{NTR}$ trimers as well as monomers. Non specific bands (B,C) are marked by **o**.

Figure 2 Aggregated Aβ 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-Aβ as described in Experimental Procedures. Immunoprecipitation with anti $p75^{NTR}$ antibodies followed by autoradiography showed initially a band of 230-240 kD (A) and with longer exposure an additional band of 80-90 kD (B). $p75^{NTR}$-NIH 3T3 cells (C) and rat cortical neurons (D) were stimulated with aggregated (agg) or soluble (sol) Aβ (1-40) and processed as described in Experimental Procedures. Western blot analysis revealed the formation of ~80 kD band in addition to the 230-240 kD band. The lower band appears more consistent with $p75^{NTR}$ monomer bound to Aβ monomer. Also, in cells stimulated with aggregated Aβ the band displaying $p75^{NTR}$ trimer is stronger than the comparable band in cultures stimulated with soluble Aβ. Non-specific bands (C) are marked by **o**.

Figure 3 Aggregated Aβ does not bind $p140^{trkA}$

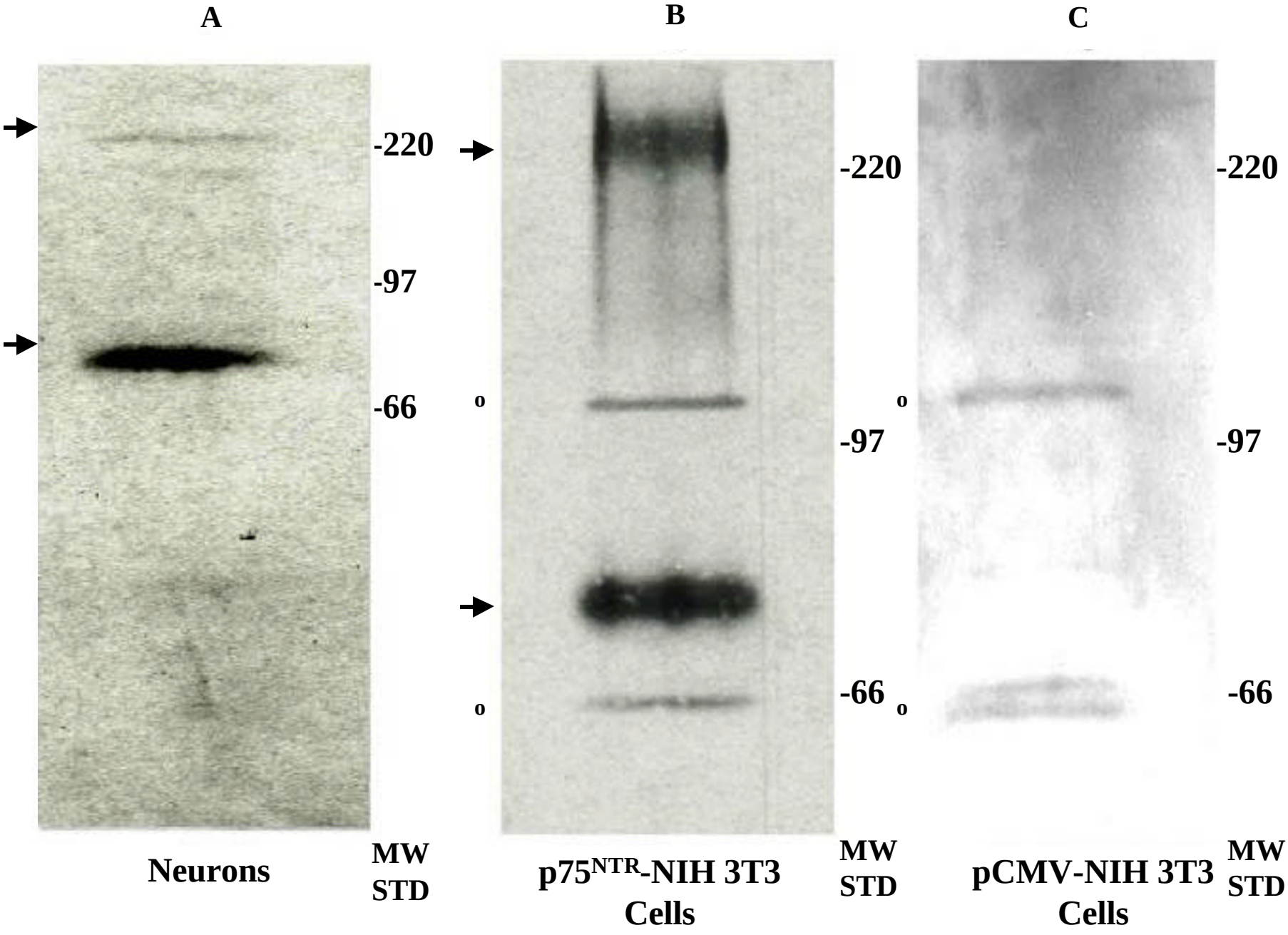
$p75^{NTR}$-NIH 3T3 cells and $p140^{trkA}$-NIH 3T3 cells were incubated for 4 hours in binding medium with 0.5 nM ^{125}I-NGF and increasing concentrations of unlabeled aggregated Aβ (0-400 nM). A concentration-dependent inhibition of ^{125}I-NGF binding by Aβ to $p75^{NTR}$-NIH 3T3 cells was observed (A). Aβ did not affect ^{125}I-NGF binding to $p140^{trkA}$-NIH 3T3 cells (B). Each point is the average of at least two separate determinations.

Figure 4 Aggregated Aβ binding to $p75^{NTR}$ induces c-jun RNA

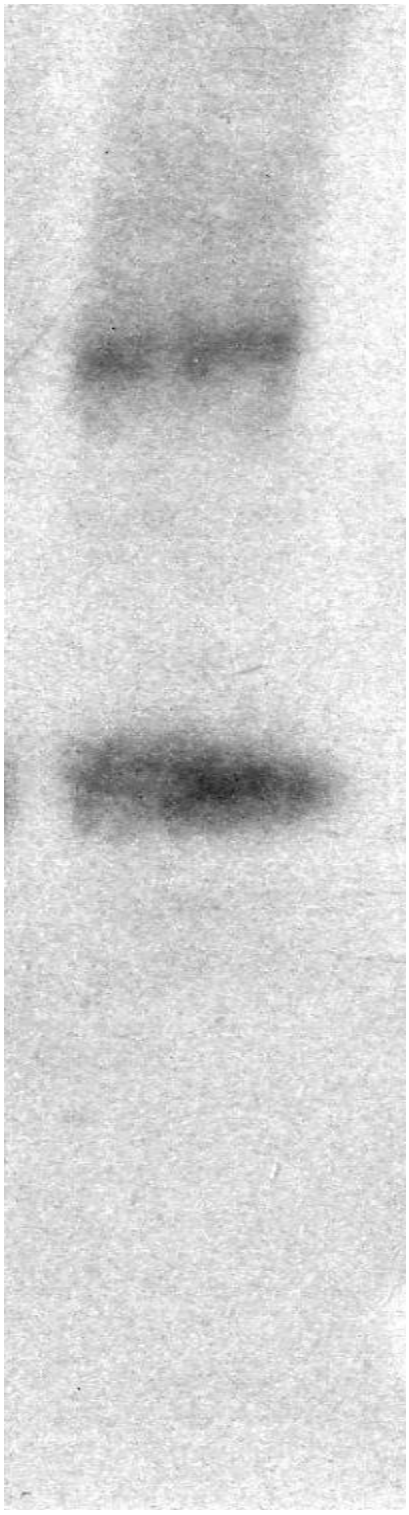
$p75^{NTR}$-NIH 3T3 cells, pCMV-NIH 3T3 (A,B) or rat cortical neurons (C,D) were stimulated with aggregated Aβ. Total cellular RNA was harvested at different intervals after stimulation and processed for northern blotting. The 2.7 and 3.2 kb c-jun transcripts were strongly induced only in $p75^{NTR}$-NIH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

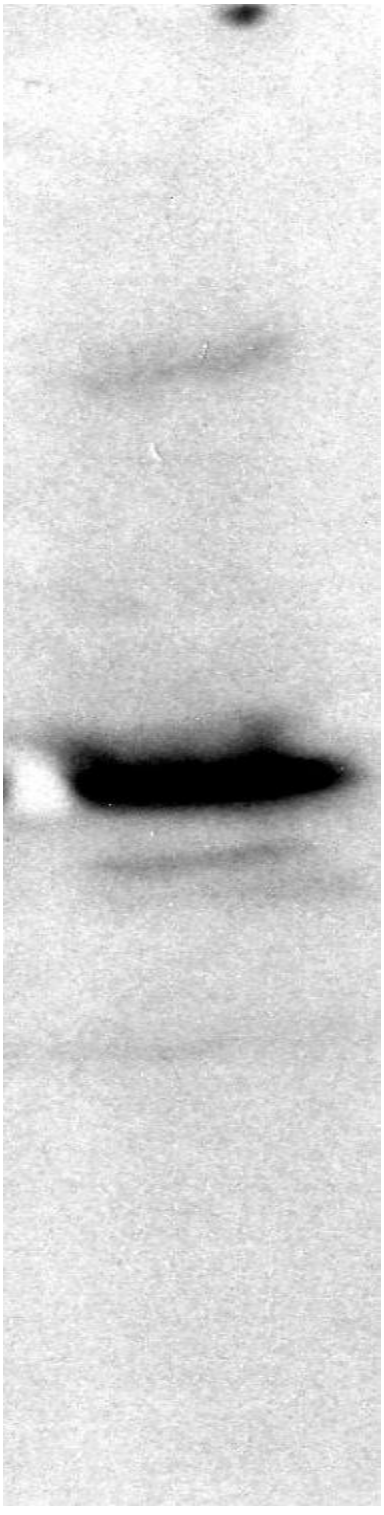
Figure 5 Aggregated Aβ binding to $p75^{NTR}$ activates JNK

(A) $p75^{NTR}$-NIH 3T3 cells and pCMV-NIH 3T3 cells were stimulated with aggregated Aβ or diluent and JNK activation was determined by phosphorylation of its substrate [GST-cJUN (1-79)]. Autoradiography demonstrates a strong band representing substrate phosphorylation in $p75^{NTR}$ NIH 3T3 cells stimulated with Aβ. (B) Graphic representation of band intensity as determined by densitometric analysis of the autoradiogram.


A
B
C
-220
-97
-66
MW STD
Neurons
p75NTR-NIH 3T3 Cells
pCMV-NIH 3T3 Cells

D





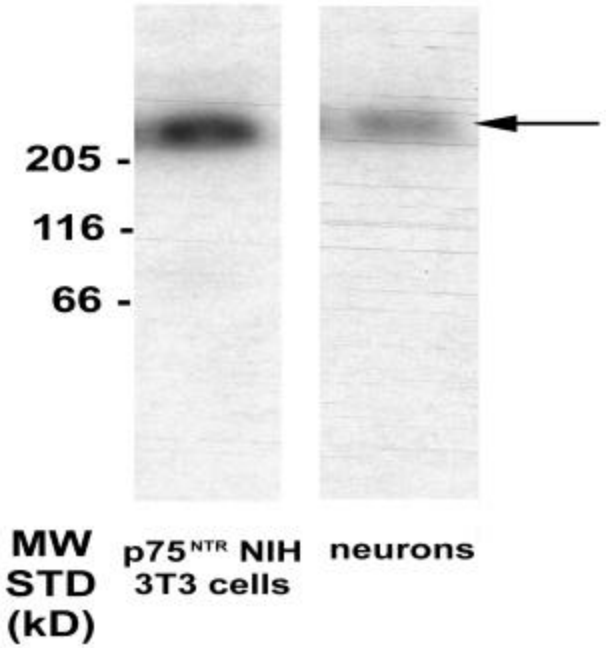
- 220

- 97

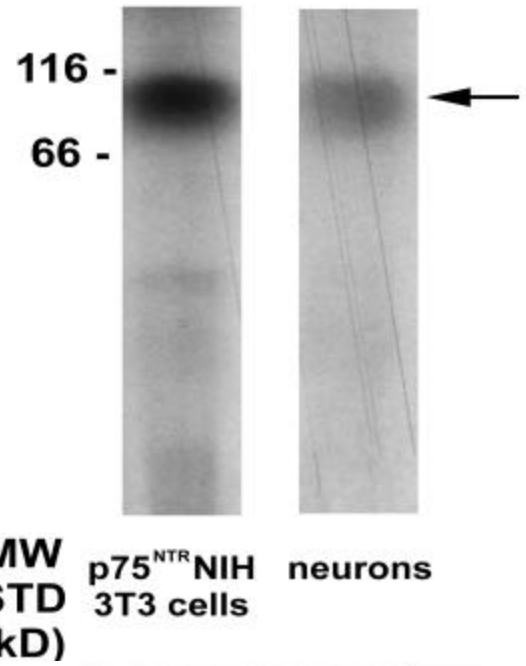
- 66

Non-reduced Reduced

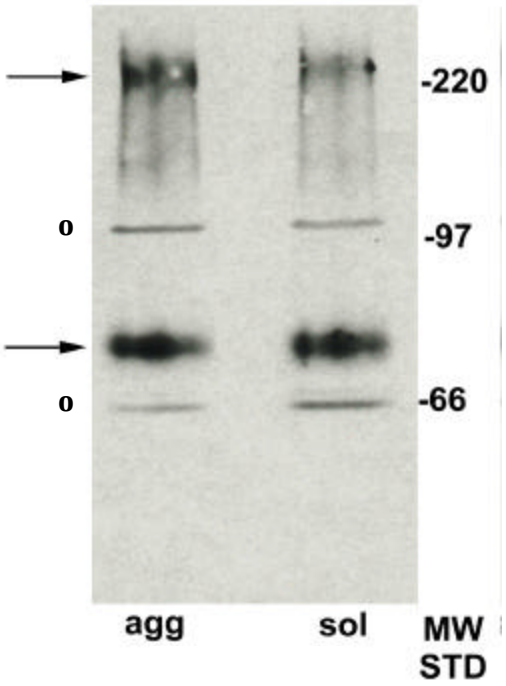
A


205 -
116 -
66 -
MW STD (kD)
p75NTR NIH 3T3 cells
neurons

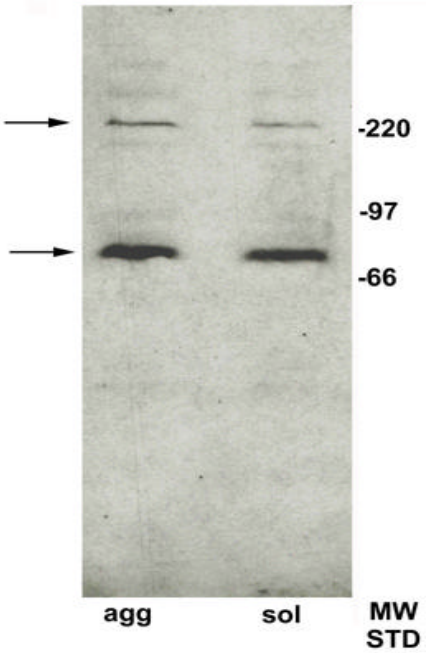
B

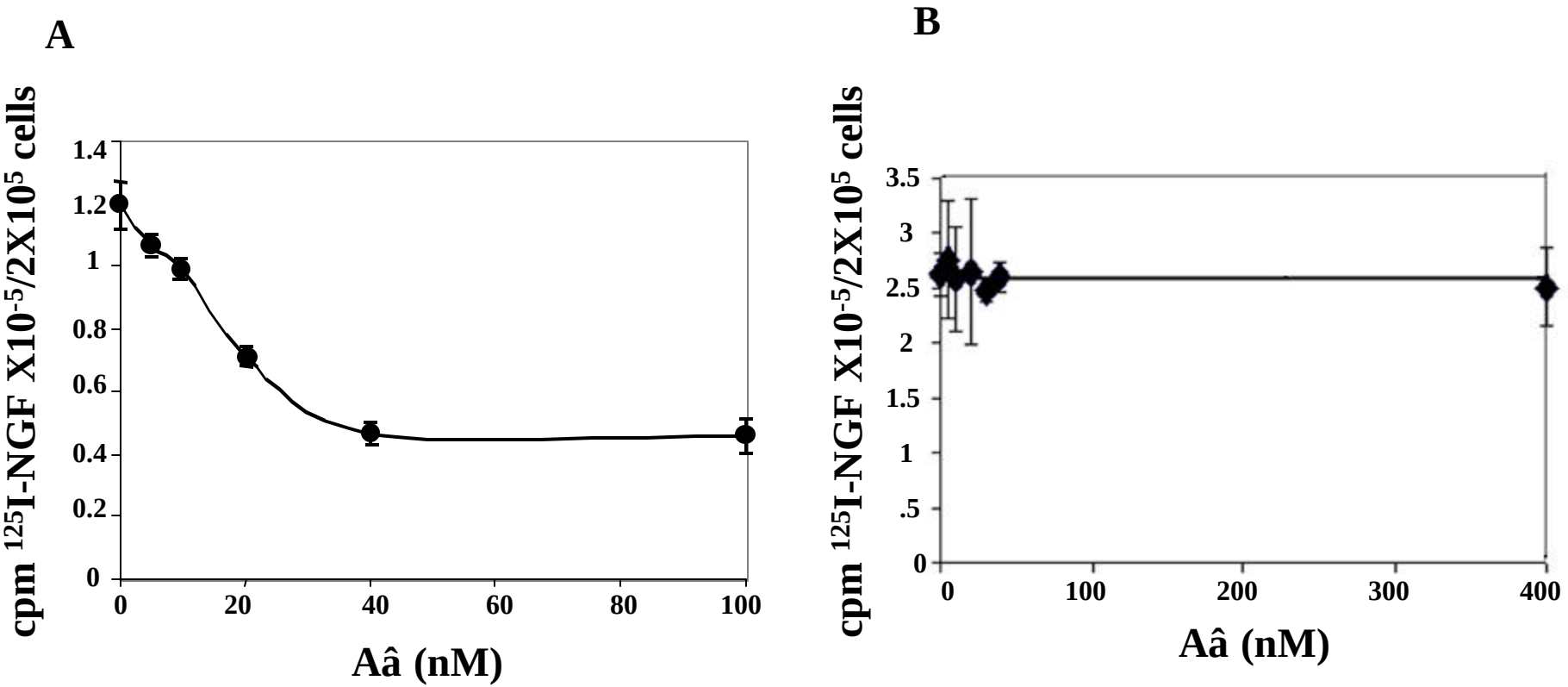

116 -
66 -
MW STD (kD)
p75NTR NIH 3T3 cells
neurons

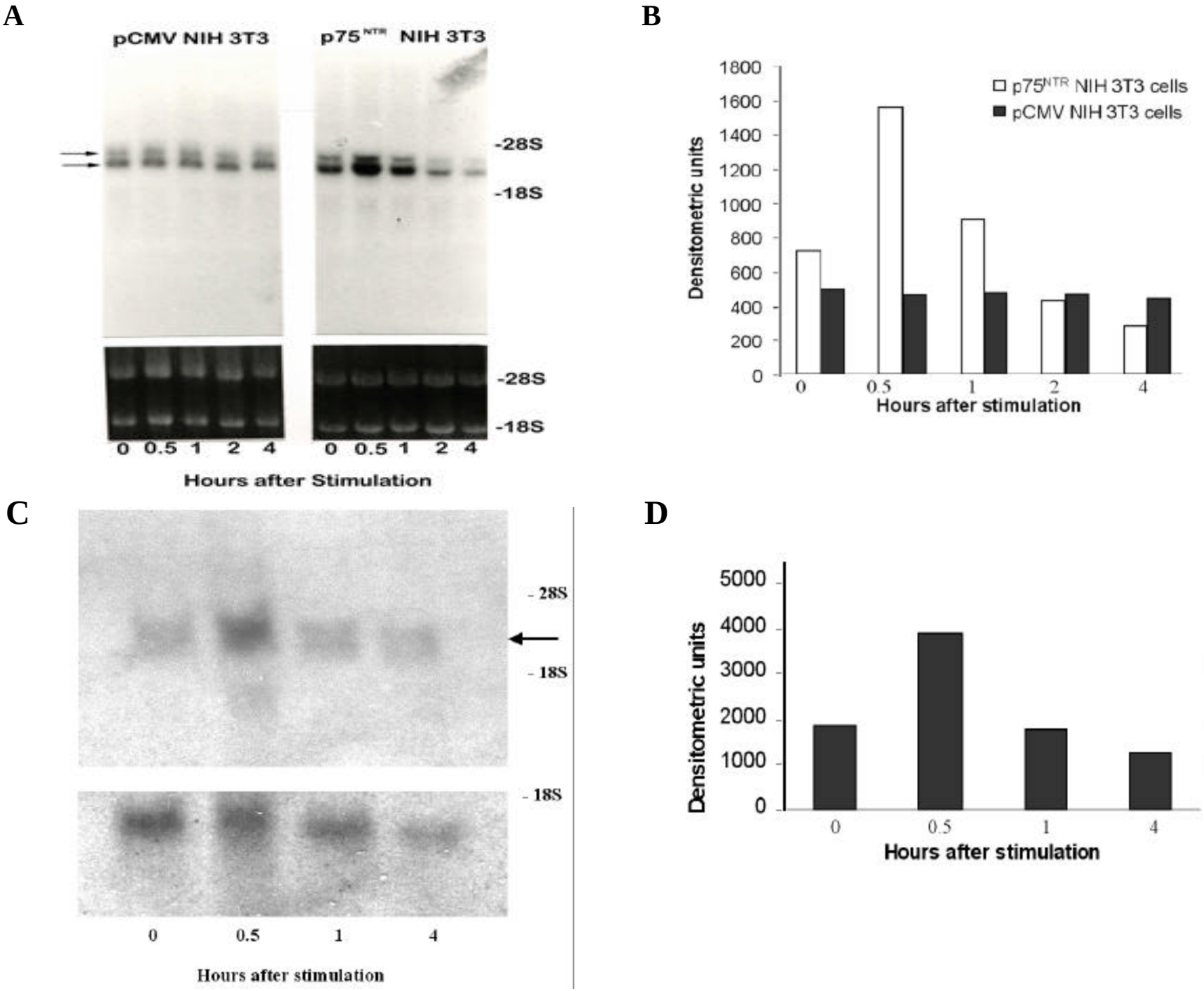
C


-220
o
-97
o
-66
agg
sol
MW STD

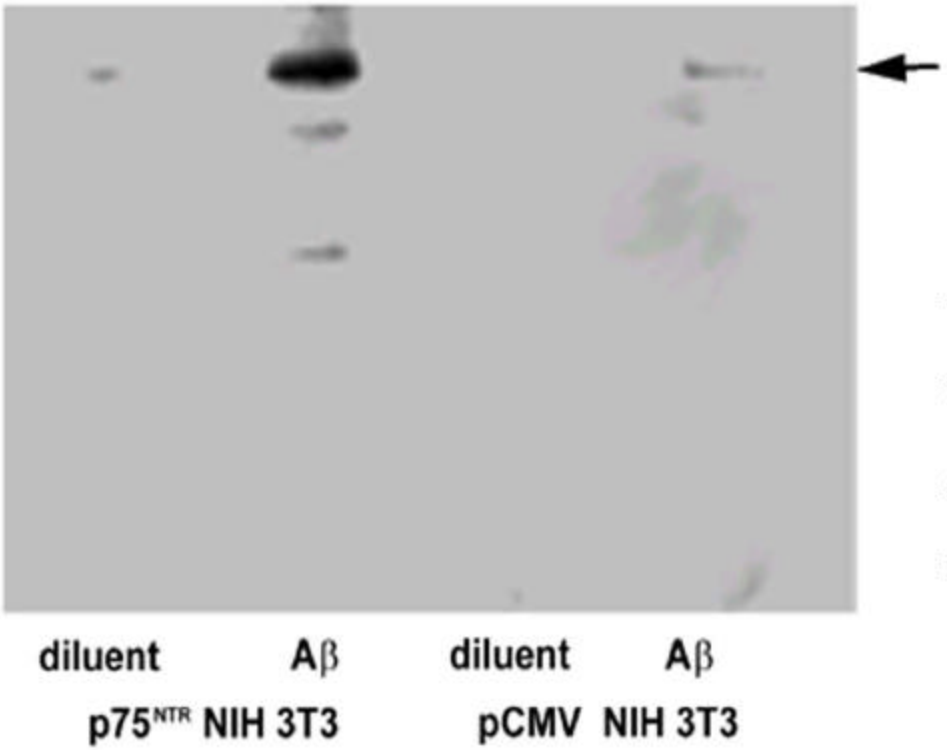
D


-220
-97
-66
agg
sol
MW STD

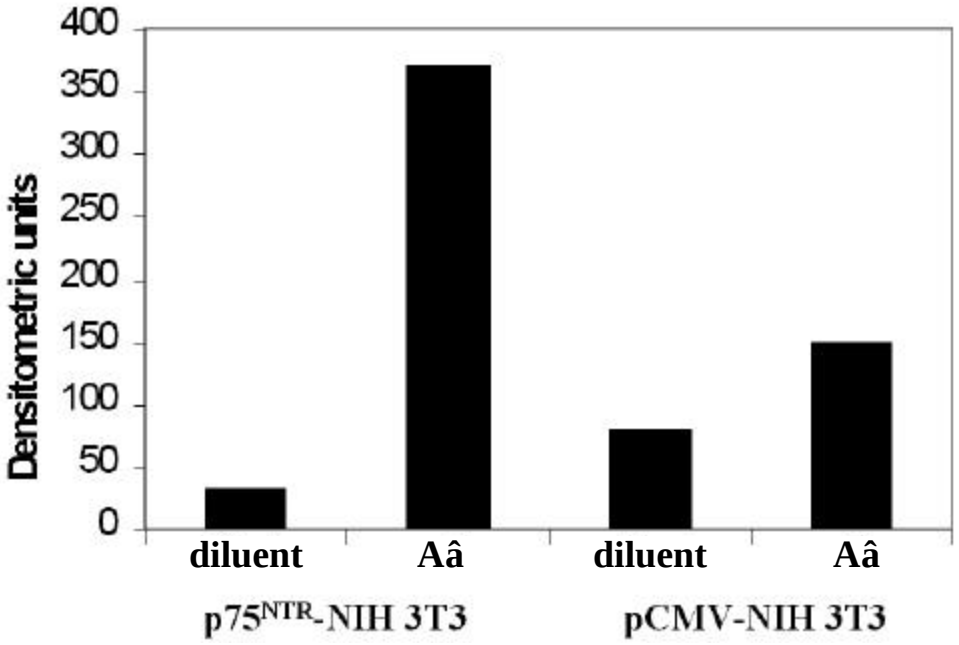

A
B
cpm 125I-NGF X10-5/2X105 cells
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
Aâ (nM)
cpm 125I-NGF X10-5/2X105 cells
3.5
3
2.5
2
1.5
1
.5
0
0
100
200
300
400
Aâ (nM)


A
pCMV NIH 3T3
p75 NTR NIH 3T3
-28S
-18S
-28S
-18S
0 0.5 1 2 4
0 0.5 1 2 4
Hours after Stimulation
B
p75NTR NIH 3T3 cells
pCMV NIH 3T3 cells
Densitometric units
1800
1600
1400
1200
1000
800
600
400
200
0
0 0.5 1 2 4
Hours after stimulation
C
- 28S
- 18S
- 18S
0 0.5 1 4
Hours after stimulation
D
Densitometric units
5000
4000
3000
2000
1000
0
0 0.5 1 4
Hours after stimulation

A


diluent
Aβ
diluent
Aβ
p75NTR NIH 3T3
pCMV NIH 3T3

B


Densitometric units
400
350
300
250
200
150
100
50
0
diluent
Aâ
diluent
Aâ
p75NTR-NIH 3T3
pCMV-NIH 3T3