Brief Communications

β-Amyloid$_{1-42}$ Induces Neuronal Death through the p75 Neurotrophin Receptor

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Alzheimer's disease is characterized by the accumulation of neurotoxic amyloidogenic peptide Aβ, degeneration of the cholinergic innervation to the hippocampus (the septohippocampal pathway), and progressive impairment of cognitive function, particularly memory. Aβ is a ligand for the p75 neurotrophin receptor (p75^{NTR}), which is best known for mediating neuronal death and has been consistently linked to the pathology of Alzheimer's disease. Here we examined whether p75^{NTR} is required for Aβ-mediated effects. Treatment of wild-type but not p75^{NTR}-deficient embryonic mouse hippocampal neurons with human Aβ_{1-42} peptide induced significant cell death. Furthermore, injection of Aβ_{1-42} into the hippocampus of adult mice resulted in significant degeneration of wild-type but not p75^{NTR}-deficient cholinergic basal forebrain neurons, indicating that the latter are resistant to Aβ-induced toxicity. We also found that neuronal death correlated with Aβ_{1-42} peptide-stimulated accumulation of the death-inducing p75^{NTR} C-terminal fragment generated by extracellular metalloprotease cleavage of full-length p75^{NTR}. Although neuronal death was prevented in the presence of the metalloprotease inhibitor TAPI-2 (tumor necrosis factor-α protease inhibitor-2), Aβ_{1-42}-induced accumulation of the C-terminal fragment resulted from inhibition of γ-secretase activity. These results provide a novel mechanism to explain the early and characteristic loss of cholinergic neurons in the septohippocampal pathway that occurs in Alzheimer's disease.

Key words: **p75^{NTR}; Alzheimer's disease; basal forebrain; cholinergic neurons; regulated intramembrane proteolysis (RIP); apoptosis; neurodegeneration**

Introduction

Alzheimer's disease is a neurodegenerative disorder characterized by senile plaque pathology, neurofibrillary tangles, and neuronal death, with resultant impairment of memory and cognitive function. Amyloid β protein (Aβ), the major component of senile plaques, is a 39–43 aa peptide produced by cleavage of the amyloid protein precursor. According to the "amyloid hypothesis," accumulation of soluble Aβ in the brain is the primary cause of the pathogenesis of Alzheimer's disease (Hardy and Selkoe, 2002), but with Aβ oligomers rather than amyloid deposits being the cause of the neurotoxicity associated with this condition (Dahlgren et al., 2002; Klein et al., 2004; Gandy, 2005).

Loss of basal forebrain cholinergic neurons that innervate the hippocampus and neocortex is an early and key feature of Alzheimer's disease (Yan and Feng, 2004; Wu et al., 2005). These cholinergic neurons express high levels of the p75 neurotrophin receptor (p75^{NTR}), which mediates cell death in a wide range of neuronal subtypes (Dechant and Barde, 2002). p75^{NTR} expression has been consistently linked to changes occurring in Alzheimer's disease (Schliebs, 2005), including the death of basal forebrain neurons (Yeo et al., 1997; Volosin et al., 2006). Like the amyloid protein precursor (APP), the extracellular domain of p75^{NTR} is cleaved by a metalloprotease, generating a transmembrane-linked C-terminal fragment. This is then cleaved by γ-secretase, liberating a soluble intracellular domain (Jung et al., 2003). Ligand binding can initiate this process, and both fragments of p75^{NTR} have been shown to mediate death signaling (Kenchappa et al., 2006; Podlesniy et al., 2006; Coulson et al., 2008; Underwood et al., 2008).

The classic p75^{NTR} ligands are the neurotrophins nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3. These are secreted as immature forms (proneurotrophins), which are sometimes cleaved to produce the mature forms (Fahnestock et al., 2001). It has previously been proposed that increased levels of proneurotrophins, such as occur in Alzheimer's disease, may underlie cholinergic neuronal degeneration (Fahnestock et al., 2001; Volosin et al., 2006). However, it is also possible that Aβ could be directly responsible for activating p75^{NTR}-mediated cell death in Alzheimer's disease, given that it is a ligand for the receptor (Yaar et al., 1997) and can stimulate p75^{NTR}-mediated death signaling cascades in cell lines (Costantini et al., 2005; Coulson, 2006). In this study, we provide evidence of a direct link between p75^{NTR} signaling and Aβ-induced

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toxicity in hippocampal neurons *in vitro* and in cholinergic basal forebrain neurons *in vivo*.

Materials and Methods

Aβ preparation. Human $A\beta_{1-42}$ peptide and control human $A\beta_{1-16}$ peptide were purchased from Dr. J Elliot (Yale University, New Haven, CT). Unless otherwise stated, peptides were dissolved in sterile water at a concentration of 200 μM and stored at 4°C (Yaar et al., 1997). Peptide solutions were incubated at 37°C for 1 h before experimental use and were found to occur in a predominantly oligomeric form (supplemental Fig. 1*A*, available at www.jneurosci.org as supplemental material), which was more toxic to hippocampal neurons than the fibrillar form (supplemental Fig. 1*B*,*C*, available at www.jneurosci.org as supplemental material).

Hippocampal cultures. All animal experiments were approved by the University of Queensland Animal Ethics Committee. When examining the effects of genotype, experiments were performed in parallel. Hippocampi were dissected from embryonic day 16 (E16) wild-type and $p75^{NTR}$-knock-out (Lee et al., 1992) C57BL/6 mice. They were then dissociated in 0.05% trypsin (Invitrogen, Melbourne, Australia), plated on 35 mm dishes coated with poly-L-lysine (0.1 mg/ml; Sigma-Aldrich, Sydney, Australia), and cultured for 24 h in a 1:1 mixture of minimal essential medium (Invitrogen) and Ham's F-12 medium (Invitrogen) supplemented with NeuroCult (StemCell Technologies, Vancouver, British Columbia, Canada). Approximately 95% of cells within the cultures were $p75^{NTR}$-positive neurons (supplemental Fig. 2*A*,*B*, available at www.jneurosci.org as supplemental material). Live cells within a defined grid were then counted ($t = 0$) under phase microscopy ($n = 4$ wells per condition per experiment). Aβ peptides or inhibitors were dissolved in fresh culture medium and applied after the $t = 0$ cell count. The final concentrations were 5 or 20 μM Aβ peptides, a 1:100 dilution of anti-extracellular $p75^{NTR}$ antibody (Ab1554; Millipore Bioscience Research Reagents, Melbourne, Australia), 200 nM compound E γ-secretase inhibitor (Calbiochem, Melbourne, Australia), and 20 μM tumor necrosis factor-α protease inhibitor-2 (TAPI-2; Peptides International, Louisville, KY). In some experiments using the metalloprotease inhibitor TAPI-2, a second 20 μM dose of TAPI-2 was added into the culture medium at $t = 6$ h. Twenty-four hours after Aβ treatment, the viability of the same neurons was again assessed, and the percentage survival was determined.

$p75^{NTR}$ cleavage in hippocampal neurons. To determine the effect of Aβ treatment on the proteolysis of $p75^{NTR}$, hippocampal neurons plated in six-well plates for 24 h were pretreated with 5 μM *β-clasto*-lactacystin (Calbiochem) proteasome inhibitor for 90 min, after which they were treated for 3 h with a mixture of 20 μM $A\beta_{1-42}$, 200 nM compound E, and/or 200 nM phorbol 12-myristate 13-acetate (PMA; Sigma-Aldrich). Cell lysates were separated using 4–20% Tris-glycine gradient gels (Invitrogen) and Western blotted using rabbit anti-human $p75^{NTR}$ intracellular domain (1:2000; Promega, Sydney, Australia). Immunoreactive bands were quantified using ImageJ software. The amount of C-terminal fragment was determined as a ratio to full-length $p75^{NTR}$ within each lane.

DNA constructs. Full-length $p75^{NTR}$ rat cDNA was as previously described (Coulson et al., 2000). A $p75^{NTR}$ C-terminal fragment mimic that is constitutively cleaved (ΔE14) was made as described by Jung et al. (2003). The C99 APP plasmid contained Gal4 DNA binding and VP16 transactivation domains fused to the carboxyl end (C99-Gal4) (Sernee et al., 2003). A ΔE14 construct was similarly modified (ΔE14-Gal4) using standard methods. The luciferase reporter gene construct (pUAS) and the constitutively active plasmid (pCMV-Gal) were as previously described (Karlstrom et al., 2002).

$p75^{NTR}$ cleavage in HEK293 cells. The effect of Aβ treatment on the proteolysis of $p75^{NTR}$ was determined using human embryonic kidney 293 (HEK293) cells grown in RPMI (Roswell Park Memorial Institute) medium and 10% fetal bovine serum. Cells were plated at 7.5×10^4 in 24-well plates and transiently transfected with 1 μg of plasmid DNA using Fugene6 (Roche, Basel, Switzerland) per well. $A\beta_{1-42}$ (16 μM) was added to the cells either 48 (overnight treatment) or 52 (3 h treatment) hours after transfection. Before cell harvest, cells were treated with *β-clasto*-lactacystin, compound E, and PMA as described above. Cell lysates were Western blotted and the results were analyzed as described above.

Luciferase cleavage assay. For luciferase experiments, HEK293 cells were plated and transfected with the pUAS plasmid together with the ΔE14-Gal4, C99-Gal4, or pCMV-Gal plasmid. The cells were then incubated overnight and/or for 3 h in Aβ or compound E, before being harvested in reporter lysis buffer. Lysis supernatant (20 μl) was assessed using the Luciferase Activity System according to the manufacturer's instructions (Promega). In each experiment, three to six replica lysates were analyzed. The total protein in each lysate was also quantified using a BCA assay kit (Pierce, Rockford, IL).

Aβ injections. Eight-week-old wild-type and $p75^{NTR}$ knock-out C57BL/6 mice were anesthetized with 8 mg/kg xylazine and 80 mg/kg ketamine by intraperitoneal injection and mounted in a stereotaxic frame. The skull was exposed and a burr hole made at stereotaxic coordinates measured from bregma (anteroposterior, −2.0 mm; mediolateral, −1.3 mm; ventral, −2.2 mm), through which 0.5 μl of saline or 100 μg/ml Aβ was injected into the CA1 region of the hippocampus at a rate of 0.5 μl/min. The needle was kept in this configuration for 2 min to prevent reflux of the injected material along the injection track. In cases in which mice were administered bilateral injections, they received $A\beta_{1-16}$ ipsilaterally and saline contralaterally, or $A\beta_{1-42}$ ipsilaterally and $A\beta_{1-16}$ contralaterally. Animals were perfused with 4% paraformaldehyde 14 d after injection.

Histology. To determine cell survival of cholinergic basal forebrain neurons, the rostral half of each brain between −1.4 and −0.5 mm from bregma (the medial septum and diagonal band of Broca) was cut by vibratome into 15 60-μm-thick serial coronal sections. Floating sections were stained with a rabbit anti-choline acetyltransferase (ChAT) polyclonal antibody (1:500; Ab143; Millipore Bioscience Research Reagents), and all ChAT-positive neurons in the area of interest were counted based on the method of Greferath et al. (2000).

Statistical analysis. The data were analyzed by ANOVA using the Newman–Keuls multiple-comparison test. All results are expressed as mean ± SD.

Results

To investigate whether $p75^{NTR}$ is required for Aβ-induced neuronal death, we first established an *in vitro* model of toxicity in E16 embryonic hippocampal neuron cultures, which have been found to be independently sensitive to both Aβ- (Ueda et al., 1994) and $p75^{NTR}$-mediated death (Troy et al., 2002). We found that 24 h after plating in serum-free medium, neurons were sensitive to an overnight incubation with oligomeric 5 or 20 μM $A\beta_{1-42}$ but not 20 μM $A\beta_{1-16}$ or vehicle (Fig. 1*A*).

To test whether $p75^{NTR}$ was required for the observed neuronal death, the effect of $A\beta_{1-42}$ on hippocampal neurons isolated from $p75^{NTR}$-deficient mice was determined. In contrast to the results obtained with wild-type neurons, $A\beta_{1-42}$ failed to induce the death of $p75^{NTR}$-deficient neurons even at 20 μM (Fig. 1*A*), indicating that $p75^{NTR}$ is required for Aβ-induced death *in vitro*. An extracellular antibody to $p75^{NTR}$ also significantly inhibited $A\beta_{1-42}$-induced hippocampal cell death (Fig. 1*B*).

Given that Aβ is a known ligand for $p75^{NTR}$, the effect of $A\beta_{1-42}$ on $p75^{NTR}$ proteolytic processing was next examined. Hippocampal neurons were treated for 3 h with 20 μM $A\beta_{1-42}$ before analysis by Western blot (Fig. 2*A*). This analysis revealed that neurons exposed to $A\beta_{1-42}$ contained significantly more $p75^{NTR}$ C-terminal fragment than control neurons (Fig. 2*B*), the amount being equivalent to that found when intracellular cleavage was blocked with compound E, a γ-secretase inhibitor, for the same period of time (Fig. 2*A*,*B*). An intracellular domain fragment of $p75^{NTR}$ was not apparent. To determine whether cleavage was required for death signaling, extracellular cleavage was blocked with the metalloprotease inhibitor TAPI-2 (Jung et al.,

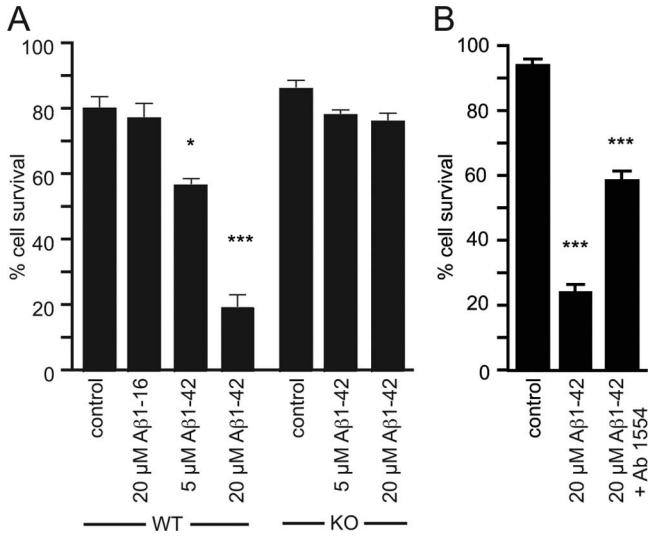

Figure 1. $A\beta_{1-42}$-induced toxicity of hippocampal neurons requires $p75^{NTR}$. ***A***, The percentage survival of cells after treatment with $A\beta_{1-42}$ or control peptide $A\beta_{1-16}$. Wild-type (WT) but not $p75^{NTR}$-deficient (KO) neurons were sensitive to $A\beta_{1-42}$-induced toxicity. $A\beta_{1-16}$ had no effect on neuronal survival. ***B***, Treatment of wild-type hippocampal neuronal cultures with a $p75^{NTR}$-blocking antibody (Ab1554) significantly inhibited $A\beta_{1-42}$ toxicity. $n = 4$ experiments. $^*p < 0.05$; $^{***}p < 0.001$.

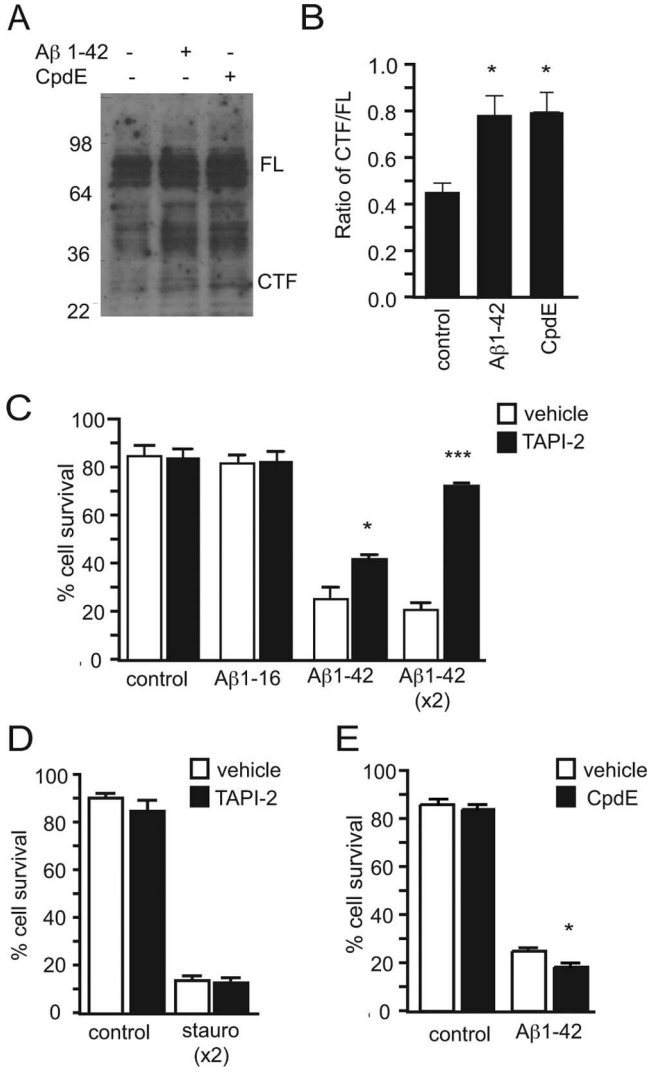

Figure 2. $A\beta_{1-42}$ toxicity regulates and requires extracellular cleavage of $p75^{NTR}$. ***A***, Anti-$p75^{NTR}$ Western blots of hippocampal neuronal cultures treated for 3 h with $A\beta_{1-42}$ and the γ-secretase inhibitor compound E (CpdE). All conditions contain β-*clasto*-lactacystin. ***B***, These treatments result in a significant increase in the amount of C-terminal fragment (CTF) as a ratio of full-length (FL) $p75^{NTR}$ as quantified by densitometry. ***C***, ***D***, $A\beta_{1-42}$ toxicity (***C***) but not staurosporine (stauro) toxicity (***D***) is significantly inhibited after treatment with the metalloprotease inhibitor TAPI-2. x2, Double TAPI-2 treatment. $n = 3$ experiments. ***E***, $A\beta_{1-42}$ toxicity is promoted in the presence of CpdE. $n = 2$ experiments. $^*p < 0.05$; $^{***}p < 0.001$.

2003). This completely inhibited $A\beta_{1-42}$- but not staurosporine-induced neuronal death in a dose-dependent manner (Fig. 2*C*,*D*). In contrast, compound E treatment resulted in a slight increase in Aβ-induced death (Fig. 2*E*).

To further examine the effect of Aβ on $p75^{NTR}$ cleavage, $p75^{NTR}$ was expressed in HEK293 fibroblast cells. Overnight treatment [but not 3 h treatment (supplemental Fig. 3*A*, available at www.jneurosci.org as supplemental material)] with $A\beta_{1-42}$ resulted in a significant increase in the amount of C-terminal fragment (Fig. 3*A*,*B*). However, in contrast to phorbol ester (PMA) treatment, there was less generation of the intracellular domain fragment of $p75^{NTR}$ (Fig. 3*C*). Interestingly, when the two treatments were combined, the resulting cleavage appeared cumulative (Fig. 3*A–C*; supplemental Fig. 3*A*, available at www.jneurosci.org as supplemental material), suggesting that their actions were independent, possibly acting on different subcellular pools of $p75^{NTR}$. Because increased amounts of C-terminal fragment could result from either increased metalloprotease activity or reduced γ-secretase activity, and coexpression of an APP γ-secretase substrate (C99) together with $p75^{NTR}$ also significantly promoted accumulation of the $p75^{NTR}$ C-terminal fragment (supplemental Fig. 2*A*, available at www.jneurosci.org as supplemental material) (Urra et al., 2007), we hypothesized that $A\beta_{1-42}$ might be competing with $p75^{NTR}$ for γ-secretase activity, rather than, or as well as, acting as a $p75^{NTR}$ ligand to promote extracellular cleavage.

Therefore, we next tested whether Aβ could alter the rate of intracellular cleavage of a $p75^{NTR}$ protein lacking the extracellular domain (ΔE14) (Jung et al., 2003). Although not as efficient as treatment with a γ-secretase inhibitor, cells treated overnight with Aβ had discernibly less intracellular domain fragment than untreated cells (Fig. 3*D*). To quantify this, we used a ΔE14 construct and a similar APP construct (C99-Gal4) in which the Gal4 transactivation domains had been fused to their carboxyl ends (Sernee et al., 2003). Only when released after γ-secretase cleavage of the C-terminal protein can the activator domain promote transcription of a cotransfected luciferase reporter gene (pUAS), the activity of which can be fluorometrically quantified in cell lysates (Karlstrom et al., 2002). Using this method, we found that overnight treatment of ΔE14-Gal4-expressing HEK293 cells with $A\beta_{1-42}$ significantly inhibited the resultant luciferase activity, whereas the control peptide $A\beta_{1-16}$ had no significant effect (Fig. 3*E*). Importantly, our results using the C99-Gal4 construct also showed a significant effect after an overnight Aβ treatment regimen, which was similar to the level of inhibition seen when the γ-secretase inhibitor was applied overnight (Fig. 3*F*). Given that the assay is based on a transcriptional readout, it was not surprising that no significant effects were seen after the 3 h treatments. These results indicate that $A\beta_{1-42}$ can generically inhibit γ-secretase activity, and suggest that the increased level of $p75^{NTR}$ C-terminal fragment in cells treated with Aβ is, at least partially, caused by this effect.

Loss of basal forebrain cholinergic neurons through degeneration of the septohippocampal pathway is a major feature of

Alzheimer's disease. To determine whether $A\beta_{1-42}$-induced toxicity in cholinergic neurons innervating the hippocampus was mediated by $p75^{NTR}$, $A\beta_{1-42}$ or $A\beta_{1-16}$ peptide solution was injected into the hippocampus of wild-type and $p75^{NTR}$-deficient mice. Fourteen days after the injection, the septohippocampal pathway was examined histologically. Regardless of the injected solution or mouse genotype, the needle track through the CA1 region of the hippocampus was identified, revealing no obvious difference in the amount of tissue damage in this area (supplemental Fig. 4, available at www.jneurosci.org as supplemental material). In contrast, the number of ChAT-positive neurons in the medial septum and diagonal band of Broca after $A\beta_{1-42}$ exposure was affected by genotype. First, the number of ChAT-positive neurons in the diagonal band of Broca of the ipsilateral hemisphere of wild-type mice injected with saline ($n = 4$, data not shown) or $A\beta_{1-16}$ was not significantly different from that found in uninjected hemispheres (Fig. 4*A*). However, the number of ChAT-positive neurons in the same region of mice injected with $A\beta_{1-42}$ was significantly reduced compared with that of uninjected, saline-injected, or $A\beta_{1-16}$-injected animals (Fig. 4*A*,*B*). Most importantly, however, the number of ChAT-positive neurons in $p75^{NTR}$-deficient mice after injection with $A\beta_{1-16}$ or $A\beta_{1-42}$ did not differ significantly, and was similar to that found in uninjected wild-type mice (Fig. 4*A*,*C*). These results demonstrate that $p75^{NTR}$ is also required for $A\beta_{1-42}$-mediated neuronal toxicity *in vivo*.

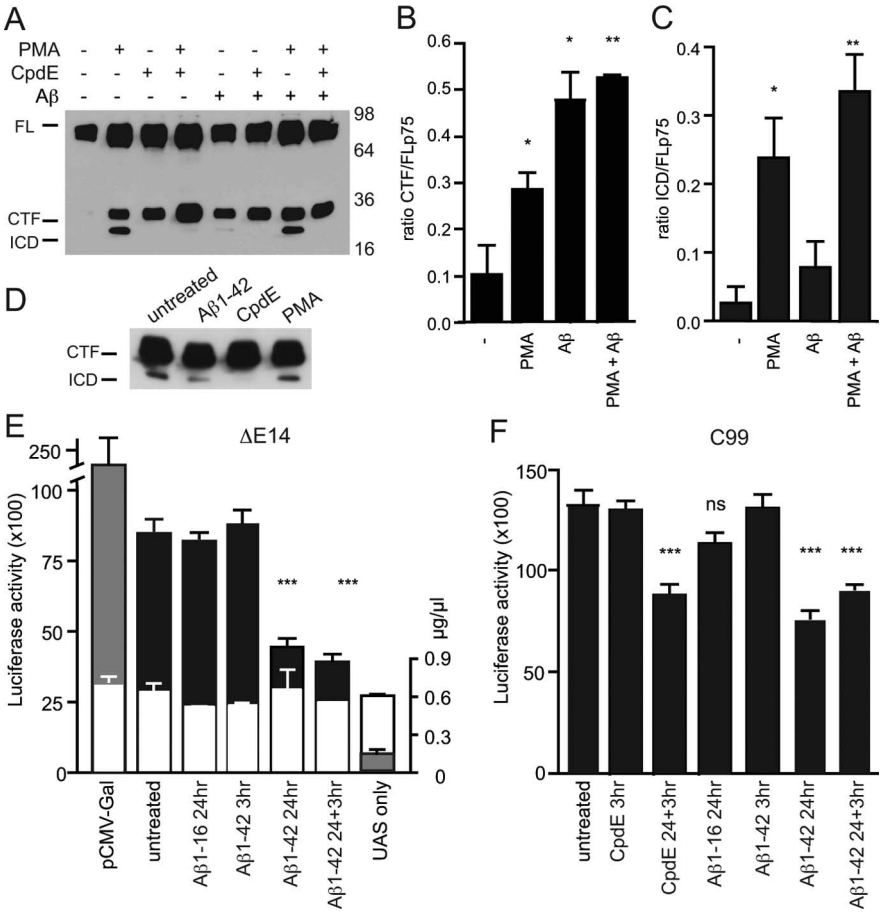

Figure 3. $A\beta_{1-42}$ inhibits γ-secretase cleavage of $p75^{NTR}$. ***A–C***, Western blot (***A***) and quantification of the amount of the C-terminal fragment (CTF; ***B***) and intracellular domain fragments (ICD; ***C***) of $p75^{NTR}$ in lysates of HEK293 cells transfected with full-length (FL) $p75^{NTR}$ before overnight treatment with $A\beta_{1-42}$ and/or 3 h treatment with PMA and/or compound E (CpdE). All cultures were treated with β-*clasto*-lactacystin. ***D***, Western blot of $p75^{NTR}$ C-terminal fragment mimic ΔE14 in lysates of transfected HEK293 cells after overnight treatment with $A\beta_{1-42}$ or 3 h treatment with CpdE or PMA. ***E***, ***F***, Luciferase activity in lysates of HEK293 cells coexpressing ΔE14-Gal4 (***E***) or C99-Gal4 (***F***) together with pUAS (black bars) after treatment with $A\beta_{1-16}$, $A\beta_{1-42}$, or CpdE. Gray bars indicate positive-control (pCMV-Gal- and pUAS-transfected cells) and negative-control (cells transfected with pUAS alone) conditions. The total amount of total protein in lysates (white bars; in micrograms per microliter) is also shown in ***E*** ($n = 6$ samples from 2 experiments). $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. $n = 4$ experiments for all other conditions.

Discussion

Here we provide evidence that $p75^{NTR}$ is required for oligomeric $A\beta_{1-42}$-mediated neuronal death *in vitro* and *in vivo*, further strengthening the case that $p75^{NTR}$ plays a role in the etiology of Alzheimer's disease.

We found that embryonic hippocampal neurons, which, unlike their adult counterparts, express high levels of $p75^{NTR}$, were killed by bathing the cultures with oligomeric $A\beta_{1-42}$ peptides, but were unaffected by $A\beta_{1-16}$ peptides. This finding is in agreement with many other studies showing that the carboxyl amino acids of the amyloid peptide are important for neuronal toxicity (Klein et al., 2004; Costantini et al., 2005), and supports the more recent idea that the soluble oligomeric protofibril form of $A\beta_{1-42}$ mediates amyloid neurotoxicity (Dahlgren et al., 2002; Gandy, 2005). Moreover, we demonstrated that $A\beta_{1-42}$-mediated neurotoxicity is abolished in the absence of $p75^{NTR}$ expression. This confirms the involvement of $p75^{NTR}$-mediated signaling pathways in neuronal death reported in previous studies using non-primary cell cultures treated with amyloid peptides (Coulson, 2006).

We found that Aβ-mediated death signaling resulted in, and required, increased production of the $p75^{NTR}$ C-terminal fragment. It has been demonstrated that Aβ binds to the extracellular domain of $p75^{NTR}$ (Yaar et al., 1997), and that $p75^{NTR}$ mediates death signaling in hippocampal neurons after neurotrophin application (Troy et al., 2002). We found that an antibody to the $p75^{NTR}$ extracellular domain had a significant effect on $A\beta_{1-42}$-induced death. It is therefore possible that the interaction of $A\beta_{1-42}$ with $p75^{NTR}$ promotes extracellular cleavage, such as occurs in response to other $p75^{NTR}$ ligands (Kenchappa et al., 2006; Podlesniy et al., 2006; Coulson et al., 2008). Although it is also a possibility that the antibody was preventing the binding and activation of $p75^{NTR}$ by endogenously produced neurotrophins (Troy et al., 2002; Volosin et al., 2006), the fact that γ-secretase inhibitor treatment failed to promote cell death in the absence of $A\beta_{1-42}$ suggests that additional $A\beta_{1-42}$ effects may play a role in the promotion of cell death.

Our experiments using HEK293 cells indicate that inhibition of γ-secretase activity is a significant factor in promoting the accumulation of the C-terminal fragment. Although the exact mechanism by which this occurs is not known, our finding that APP processing is also affected by Aβ, together with the demonstration that increased expression of the APP C-terminal frag-

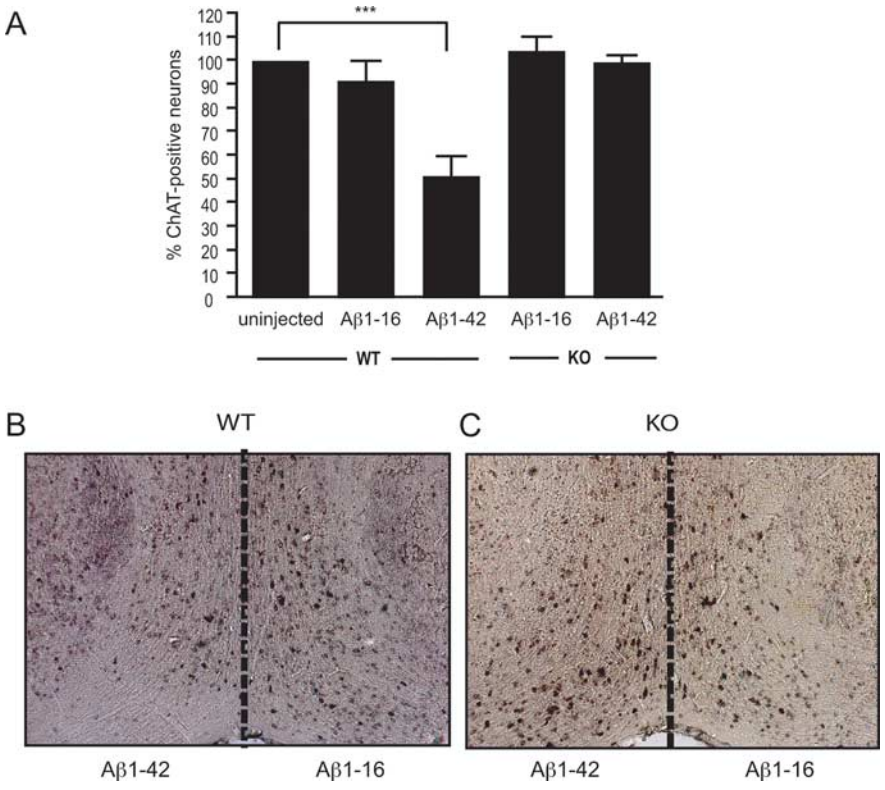

Figure 4. $A\beta_{1-42}$ toxicity of basal forebrain cholinergic neurons *in vivo* is prevented by $p75^{NTR}$ deficiency. The hippocampi of wild-type (WT) and $p75^{NTR}$ knock-out (KO) mice were injected with Aβ, and after 14 d the number of ChAT-positive cells in each hemisphere was counted. ***A***, The number of wild-type ChAT-positive neurons in the hemisphere ipsilateral to the $A\beta_{1-42}$ injection site was significantly reduced compared with that in uninjected or $A\beta_{1-16}$-injected animals. The numbers of ChAT-positive neurons in $p75^{NTR}$-deficient mice after injection with $A\beta_{1-16}$ or $A\beta_{1-42}$ were not significantly different, and were similar also to that found in uninjected wild-type mice. $n \geq 4$ per condition; $^{***}p < 0.001$. ***B***, ***C***, Photomicrographs of ChAT-positive cells in the basal forebrain of wild-type (***B***) and $p75^{NTR}$-deficient (***C***) mice 14 d after injection with Aβ.

ment has a similar effect on $p75^{NTR}$ processing, suggests that $A\beta_{1-42}$ could act as a competitive γ-secretase substrate. This mode of action, in agreement with our finding that γ-secretase inhibitor treatment promoted rather than inhibited Aβ-induced neuronal death, would preclude Aβ-induced cell death being mediated via the intracellular domain pathway described by others (Kenchappa et al., 2006; Podlesniy et al., 2006). However, we have previously shown that the C-terminal fragment of $p75^{NTR}$ is constitutively active in signaling neuronal death, and have recently defined a signaling cascade by which it results in caspase activity (Coulson et al., 2000, 2008; Underwood et al., 2008).

In support of $p75^{NTR}$ playing a key role in $A\beta_{1-42}$-induced neurodegeneration, we found that injection of $A\beta_{1-42}$ into the hippocampus of wild-type mice, a model of Alzheimer's disease, resulted in the degeneration of ChAT-positive forebrain neurons *in vivo*. Although our analysis did not specifically determine that ChAT-positive cells had died after $A\beta_{1-42}$ exposure at axonal terminals, it did demonstrate the loss of cellular neurotransmitter production and hence loss of function of these neurons. It has also been shown in aged animals that ChAT downregulation correlates with loss of hippocampal function (Yeo et al., 1997) and that this precedes the death of basal forebrain neurons (Greferath et al., 2000).

This functional loss of cholinergic forebrain neurons *in vivo* was also prevented by $p75^{NTR}$ deficiency, supporting the hypothesis that $p75^{NTR}$-mediated degeneration of basal forebrain neurons is an early consequence of increased oligomeric Aβ levels and is tightly linked to the loss of septohippocampal function observed in both animal models and human patients with Alzheimer's disease (Klein et al., 2004; Gimenez-Llort et al., 2007). Although a significant difference in the numbers of ChAT-positive basal forebrain neurons correlated directly with $A\beta_{1-42}$ exposure, we found no obvious difference in the amount of damage in the hippocampus after injections into the CA1 region, regardless of Aβ peptide length or genotype. In the adult hippocampus, unlike the embryonic hippocampus and the adult basal forebrain, $p75^{NTR}$ is downregulated, although it can be upregulated in response to injury (Dechant and Barde, 2002). This observation further highlights the connection between the degeneration of neurons that express high levels of $p75^{NTR}$ and $A\beta_{1-42}$ peptide exposure. Although our *in vitro* results demonstrate that $A\beta_{1-42}$-induced, $p75^{NTR}$-mediated neuronal death is likely to result from inhibition of γ-secretase activity, other injury-induced factors, such as modulation of synaptic plasticity (Klein et al., 2004), and/or neurotrophins released by reactive astrocytes (Pehar et al., 2004), could also contribute to, and indeed may be required to, promote cell death *in vivo* via a $p75^{NTR}$-dependent mechanism.

In conclusion, we have shown that $A\beta_{1-42}$-induced neuronal degeneration of embryonic hippocampal neurons *in vitro* and basal forebrain neurons *in vivo* requires $p75^{NTR}$. Furthermore, the activation of neuronal loss strongly correlates with increased accumulation of the constitutively active death-signaling C-terminal fragment of $p75^{NTR}$ generated by inhibition of γ-secretase activity. Although $p75^{NTR}$ has been causally linked to the progression of Alzheimer's disease for over a decade, the current results provide a novel mechanism to explain the early and characteristic degeneration of the septohippocampal pathway that occurs in this disease.

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