The p75 Neurotrophin Receptor Activates Akt (Protein Kinase B) through a Phosphatidylinositol 3-Kinase-dependent Pathway*

The Akt kinase plays a crucial role in supporting Trk-dependent cell survival, whereas the p75 neurotrophin receptor (p75NTR) facilitates cellular apoptosis. The precise mechanism that p75NTR uses to promote cell death is not certain, but one possibility is that p75NTR-dependent ceramide accumulation inhibits phosphatidylinositol 3-kinase-mediated Akt activation. To test this hypothesis, we developed a system for examining p75NTR-dependent apoptosis and determined the effect of p75NTR on Akt activation. Surprisingly, p75NTR increased, rather than decreased, Akt phosphorylation in a variety of cell types, including human Niemann-Pick fibroblasts, which lack acidic sphingomyelinase activity. The p75NTR expression level required to elicit Akt phosphorylation was much lower than that required to activate the JNK pathway or to mediate apoptosis. We show that p75NTR-dependent Akt phosphorylation was independent of TrkA signaling, required active phosphatidylinositol 3-kinase, and was associated with increased tyrosine phosphorylation of p85 and Shc and with reduced cytosolic tyrosine phosphatase activity. Finally, we show that p75NTR expression increased survival in cells exposed to staurosporine or subjected to serum withdrawal. These findings indicate that p75NTR facilitates cell survival through novel signaling cascades that result in Akt activation.

The neurotrophins are a family of growth factors involved in the survival, development, and death of specific populations of neurons and non-neuronal cells. Nerve growth factor (NGF),¹ the prototypic neurotrophin, is the best characterized member of this family, which in mammals, also includes brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5 (1). The signal transduction systems that mediate the diverse biological functions of the neurotrophins are initiated by two categories of cell-surface receptors: the Trk receptors and the p75 neurotrophin receptor (p75NTR).

One of the main survival pathways for neuronal cell survival is mediated by phosphatidylinositol 3-kinase (PI3K) and involves activation of the Akt serine/threonine kinase (2). Increased phosphatidylinositol 3,4,5-trisphosphate (PIP₃) production results primarily from relocation of PI3K from the cytosol to a juxtamembrane location that provides access to PIP substrates. This redistribution of PI3K requires the association of the SH2 domain within the p85 regulatory subunit of PI3K with phosphorylated tyrosines present on activated cell-surface receptors or on receptor-associated adaptor proteins (reviewed in Ref. 3). Accumulation of PIP₃ and its phospholipid phosphatase product, phosphatidylinositol 3,4-bisphosphate, in the plasma membrane creates docking sites for the pleckstrin homology domains of phosphoinositide-dependent kinase-1 and Akt. Phosphorylation of Akt on threonine 308 by phosphoinositide-dependent kinase-1 followed by autophosphorylation on serine 473 activates Akt (4, 5) and allows the enzyme to facilitate survival by phosphorylation of downstream substrates that may include Bad, Caspase-9, Forkhead family members, IκB kinase, and glycogen synthase kinase-3 (6–12).

p75NTR binds all neurotrophins with similar affinity and is a member of the tumor necrosis factor receptor (TNFR) superfamily (13, 14). Current data suggest that the main physiological functions of p75NTR are to regulate Trk receptor activation and signaling (15–19) and to activate Trk-independent signal transduction cascades involving sphingomyelinase (20–22), nuclear factor-κB (23–25), and JNK (26–28). Several findings indicate that NGF binding to p75NTR can initiate a cell death cascade in some cell types. For example, NGF treatment of embryonic retinal cells or postnatal oligodendrocytes that express p75NTR, but not TrkA, increases cellular apoptosis (29–31). The precise signaling pathway(s) used by p75NTR to activate cell autonomous death cascades remain unclear, but may involve activation of caspase-1, -2, and -3 (32) as well as cyclin-dependent kinases (33). A number of cytosolic proteins that interact directly with the p75NTR intracellular domain have been identified, including TRAF proteins (25, 34), caveolin (35), SC-1 (36), NRIF (37), NADE (39), RhoA (40), and NRGAGE (41), but linking each of these to precise p75NTR signaling cascades remains a major challenge.

Activation of cell death cascades can result from suppression of signaling pathways that normally support survival. In some systems, sphingomyelinase activation results in a ceramide-dependent decrease in the generation of PIP₃ and a subsequent reduction in Akt activity (42, 43); and in others, ceramide
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reduces Akt activity through specific dephosphorylation of serine 473 (44). Since p75NTR activates sphingomyelinase in a neurotrophin-dependent manner, we have determined if p75NTR activation can suppress Akt and thereby facilitate apoptosis. Our results show that p75NTR does indeed regulate Akt; but contrary to our expectations, we found that p75NTR increases Akt activation through a Trk-independent pathway that requires PI3K and show that p75NTR expression suppresses apoptosis. Although high levels of p75NTR will mediate cell death, the p75NTR expression level required to elicit Akt phosphorylation is much lower than that required to activate the JNK pathway or to mediate apoptosis. The effect of p75NTR on Akt correlates with increased tyrosine phosphorylation of the p85 regulatory subunit of PI3K and of Nc3 adapter proteins, suggesting that PTPase inhibition may play a role in this effect. Consistent with this, p75NTR expression results in reduced cytosolic tyrosine phosphatase activity. These data indicate that a physiological role of p75NTR is to enhance cell survival through an Akt-dependent pathway.

EXPERIMENTAL PROCEDURES

Materials—NGF was purchased from Collaborative Research (Bedford, MA); cell culture reagents were from BioWhittaker, Inc. (Walkersville, MD); and all other reagents were from Sigma, ICN Biochemicals (Costa Mesa, CA), or Calbiochem unless otherwise indicated.

Preparation of Recombinant Adenoviruses—pAd-CMV5-F1 containing full-length rat p75NTR (45), the p75NTR intracellular domain (46), or the p75NTR intracellular domain modified to contain an N-terminal myristoylation tag derived from Hck, an Src-related kinase (47), was cotransfected with replication-defective adenoviral DNA (Quantum Biotechnologies, Laval, Quebec, Canada) into 293A cells. Crude virions derived from viral plagues were used to infect 293A cells. p75NTR expression was confirmed by immunoblot analysis, and positive plaques were purified twice by limiting dilution. Recombinant adenoviruses were amplified in 293A cells, purified on sucrose gradients, and titrated by plaque assay in 293A cells. Control recombinant adenoviruses expressing β-galactosidase (lacZ) or green fluorescent protein were generated as described previously (24).

Immunoblotting—Immunoblotting for total and phosphorylated proteins was performed using rabbit polyclonal antibodies from New England Biolabs, Inc. (Beverly, MA) or from Upstate Biotechnology, Inc. (Lake Placid, NY). p75NTR immunoreactivity was detected using p75NTR-B1, a rabbit polyclonal antibody directed against a glutathione S-transferase fusion protein containing amino acids 276–425 of the intracellular domain of rat p75NTR (Babco, Berkeley, CA) (46, 48). Protein content from cell lysates was normalized using the BCA assay (Pierce), and 10–25 μg of protein was solubilized in Laemmli sample buffer (49), separated by SDS-polyacrylamide gel electrophoresis, and electroblotted onto nitrocellulose. Blocking and secondary antibody incubations of immunoblots were performed in Tris-buffered saline/Tween (10 mM Tris (pH 7.4), 150 mM NaCl, and 0.2% Tween 20) supplemented with 5% (w/v) dried skim milk powder. Primary antibody incubations were performed in Tris-buffered saline/Tween supplemented with 5% bovine serum albumin. For 4G10, 2% (w/v) bovine serum albumin was used for the blocking step. Horseradish peroxidase-conjugated donkey anti-rabbit IgG, horseradish peroxidase-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA), and wheat germ agglutinin (Amersham Pharmacia Biotech) precipitation, and equivalent amounts were analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide Survival Assays—Analysis of cell survival was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, which was added at a final concentration of 1 ng/ml for 4 h following a 48-h infection. The reaction was ended by the addition of 1 volume of solubilization buffer (20% SDS, 10% dimethylformamide, and 20% acetic acid). After overnight solubilization, specific and nonspecific absorbances were read at 570 and 630 nm, respectively. Each condition was tested six times, and results were analyzed for statistical significance by multiple analysis of variance.

Apoptotic Assays—Apoptotic cell death was quantified using annexin V binding and FACScan analyses. Briefly, cells were harvested using PBS with 2 mM EDTA and washed twice in PBS supplemented with 2% BSA. For the annexin V assay, cells were resuspended in 0.1 ml of PBS containing 1 μg/ml fluorescein isothiocyanate-conjugated annexin V (Becton Dickinson, Mountain View, CA) and incubated for 15 min in the dark at room temperature. PBS (0.3 ml) was added to each tube, and cells were analyzed on a FACScan flow cytometer (Becton Dickinson-Pharmingen). For FACScan analyses of cells with sub-G1 DNA content, cells were harvested and resuspended in 50% ethanol and PBS and left on ice for 30 min. An underlayer of 1 volume of PBS containing 2% bovine serum albumin and 2% BSA and then incubated for 30 min in blocking buffer supplemented with 1:500 dilution of fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch Laboratories, Inc.). Cells were washed three times and resuspended in 0.1 ml of PBS with 0.1 mg/ml RNase A for 15 min at room temperature. PBS (0.3 ml) with 25 μg/ml propidium iodide was added to the cells, incubated for 15 min, and then analyzed on a FACScan. Each condition was tested in triplicate, and results were analyzed for statistical significance by multiple analysis of variance.

Protein-Tyrosine Phosphatase Assays—Twenty-four hours after infection, PC12nrr5 cells were washed twice in cold HEPES-buffered saline to remove free phosphate and then harvested in 1 ml of suspension buffer (50 mM HEPES, 150 mM NaCl, 2 mM EDTA, 0.25 mM sucrose, 1 mM dithiothreitol, 10 μg/ml leupeptin, 25 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride). Sonicated, and spun at 1000 × g for 5 min to remove intact mitochondria and nuclei. Cytosolic and membrane fractions were separated by spinning at 100,000 × g for 60 min at 4 °C. The membrane pellet was resuspended for 30 min in 1 ml of suspension buffer supplemented with 1% Triton X-100 and then spun at 1000 × g for 5 min to remove insoluble contaminants. Endogenous phosphate was removed from membrane and cytosolic fractions by buffer exchange.

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FIG. 1. Overexpression of p75NTR in PC12nnr5 cells induces cell death. A, PC12nnr5 cells were infected with recombinant adenoviruses expressing LacZ, full-length p75NTR, the intracellular domain of p75NTR (p75ICD), or the myristoylated p75NTR intracellular domain (p75mICD) at increasing multiplicities of infection and, after 48 h, were assayed for survival using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described under “Experimental Procedures.” B, p75mICD is enriched in cell membranes, but p75ICD is cytosolic. COS-7 cells were transiently transfected with plasmids encoding either p75ICD (ICD) or p75mICD (mICD) and, after 48 h, fractionated as described under “Experimental Procedures.” C indicates control. C, PC12nnr5 cells that were infected and incubated under the same conditions as described for A were lysed and analyzed for c-Jun (phospho-Ser73 (pS73)), JNK (phospho-Thr183/Tyr185), and CREB (phospho-Thr133) phosphorylation by immunoblotting. Results shown in A are represented as the means ± S.E. of three independent experiments, and the experiment in C was repeated twice with similar results.

Recombinant Adenoviruses Encoding p75NTR Activate JNK and Induce Cell Death of PC12nnr5 Cells—The actions of p75NTR are complex and not well understood. This is due in part to the lack of cellular systems in which p75NTR-dependent signaling events can be reliably observed. Previous work from our group (46) and others (50) has shown that overexpression of the intracellular domain of TNFR superfamily members will constitutively activate downstream signaling pathways. To reliably activate p75NTR signaling cascades in a variety of cellular circumstances, recombinant adenoviruses encoding full-length p75NTR, the intracellular domain of p75NTR (p75ICD), or a myristoylated form of the intracellular domain of p75NTR that is targeted to the plasma membrane (p75mICD) (Fig. 1B) were produced. Recombinant adenoviruses encoding LacZ or green fluorescent protein were used as controls for all studies. To validate signaling properties of these p75NTR recombinant viruses, we initially tested their effects on the survival of PC12nnr5 cells (which express endogenous p75NTR, but not TrkA). Fig. 1A shows that LacZ virus had no effect on cell survival, whereas viruses encoding p75NTR or p75ICD were cytotoxic at a multiplicity of infection (m.o.i.) of 200. Overexpression of p75mICD induced cytotoxicity at lower multiplicities of infection than that of p75NTR or p75ICD, suggesting that membrane localization of the intracellular domain may be important for p75NTR-dependent apoptosis. p75NTR-dependent cell death was not an artifact of protein overexpression or viral infection since equivalent quantities of LacZ adenovirus had no effect on cell survival. Several reports have shown that p75NTR activation can lead to JNK activation (26–28); and consistent with this, we found that expression of p75mICD (Fig. 1C), p75ICD, and p75NTR increased phosphorylation of JNK on threonine 183 and tyrosine 185 and increased c-Jun phosphorylation on serine 73. Equivalent levels of LacZ virus did not alter JNK or c-Jun phosphorylation (data not shown). The level of c-Jun protein was also elevated at high p75NTR expression levels, likely due to autoregulation of c-jun transcription (51) during the relatively long infection period (48 h). To demonstrate the specificity of the JNK activation initiated by p75NTR, we analyzed phosphorylation of the transcription factor CREB, which lies downstream of protein kinase A, and assessed the phosphorylation status of MKK3/6, which activates p38 MAPK. Neither CREB (Fig. 1C) nor MKK3/6 (data not shown) phosphorylation was altered by p75NTR expression. These results show that p75NTR signaling results in specific activation of the JNK pathway and promotes cell death and therefore demonstrate that the p75NTR recombinant adenoviruses show the expected constitutive signaling properties.

p75NTR Activates Akt—Recent reports have shown that ceramide inhibits PI3K activity, reduces cellular PI3K levels, and thereby inhibits Akt activity (42, 43). p75NTR activates sphingomyelinase upon neurotrophin binding (20, 21), and we hypothesized that p75NTR could facilitate apoptosis by attenuating PI3K production and reducing Akt activity. To determine if p75NTR alters Akt activity, PC12nnr5 cells were infected with recombinant virus expressing each of the three p75NTR isoforms or LacZ and analyzed for Akt activation using phospho-specific antibodies directed against Akt serine 473 (Ser473), an Akt autophosphorylation site that correlates with Akt kinase activity (5). Surprisingly, expression of full-length p75NTR, p75ICD (Fig. 2), or p75mICD (see Figs. 3B and 6B) resulted in significant increases in the phosphorylation of Akt on Ser473, whereas control adenovirus expressing LacZ had no effect on Akt Ser473 phosphorylation. As noted above, relatively high levels of p75NTR expression were required to observe JNK activation, c-Jun phosphorylation, and apoptosis, but much lower levels of p75NTR expression were sufficient to induce Akt phosphorylation. p75NTR-mediated reduction of Akt phosphorylation was never observed, even in cells exposed to high...
Expression by immunoblotting. Experiments were repeated three times for LacZ expression, and p75NTR expression by immunoblotting. Cells were harvested 24 h after infection and analyzed for Akt levels, Akt serine 473 phosphorylation, LacZ expression, and p75NTR expression. Cells were infected with recombinant adenovirus expressing LacZ, full-length p75NTR, or the intracellular domain of p75NTR (p75ICD) at different multiplicities of infection. Cells were harvested 24 h after infection and analyzed for Akt levels, Akt serine 473 phosphorylation, LacZ expression, and p75NTR expression by immunoblotting. Experiments were repeated three times with similar results.

The effects of ligand binding to p75NTR on Akt activation in the presence and absence of virus were also tested; treatment of control and infected cells with NGF, brain-derived neurotrophic factor, or neurotrophin-3 at various concentrations and time courses had no effect on the p75NTR adenovirus-induced phosphorylation of Akt in PC12nnr5 cells (data not shown). Therefore, these data indicate that p75NTR expression activates Akt in a ligand-independent manner.

p75NTR-induced Akt Phosphorylation Is Trk-independent and Does Not Require Acidic Sphingomyelinase—The TrkA receptor is a potent activator of Akt, and our previous results (15) and those of others (16, 52) have shown that p75NTR can increase the response of TrkA to limiting NGF concentrations. It was therefore possible that the p75NTR-dependent Akt phosphorylation observed is secondary to activation of low levels of TrkA that may be present in PC12nnr5 cells. To address this, PC12nnr5 cells were first examined to determine if they express NGF-responsive TrkA. Fig. 3A shows that activated TrkA was not detected in PC12nnr5 cells under conditions in which TrkA was readily detected in normal PC12 cells. We then tested K252a, a specific TrkA inhibitor, for its ability to block p75NTR-mediated Akt phosphorylation using recombinant viruses encoding p75ICD and p75mICD, which are incapable of binding ligand. LacZ, p75ICD, and p75mICD adenoviruses were used at m.o.i. = 25 in these experiments since this infection level activated Akt (Fig. 2), but did not induce cell death (Fig. 1). Fig. 3B shows that K252a completely blocked NGF-mediated Akt phosphorylation in PC12 cells, but had no effect on p75ICD- and p75mICD-induced Akt phosphorylation in either PC12nnr5 or PC12 cells. Therefore, p75NTR-mediated activation of Akt occurs independently of TrkA signaling.

PC12nnr5 cells express endogenous p75NTR, and it is conceivable that p75NTR overexpression may mediate an increase in Akt phosphorylation by disrupting signaling from endogenous p75NTR. To address this, the effects of p75NTR on Akt phosphorylation were determined in a variety of cell types that do not express p75NTR or TrkA. p75NTR expression in MG87-3T3 fibroblasts (m.o.i. = 100) (Fig. 3C) or HeLa cells (data not shown) resulted in increased Akt phosphorylation, similar to that observed in PC12nnr5 cells. These results indicate that the effect of p75NTR on Akt phosphorylation does not involve disruption of endogenous p75NTR signaling.

Recombinant adenoviruses have significant effects on cellular physiology, and we therefore sought additional means to confirm that p75NTR expression increases Akt activation. TIMp75-3 is an MG87-derived cell line in which expression of p75NTR is tightly regulated through the addition of doxycycline (24) and therefore could be used to confirm that p75NTR overexpression results in Akt phosphorylation. Cells incubated with 2.5 μg/ml doxycycline for 24 h showed the expected increase in p75NTR expression, and this correlated with a rise in Akt Ser473 phosphorylation (Fig. 3D). Therefore, two independent means of p75NTR expression result in Akt activation in different cell types. The relatively modest increase in Akt phosphorylation observed likely reflects the fact that only a subpopulation of TIMp75-3 cells show robust doxycycline-induced p75NTR expression (see Fig. 5B).

Neurotrophin binding to p75NTR increases sphingomyelinase activity, and one possible explanation for the effect of Akt phosphorylation observed is secondary to activation of low levels of TrkA that may be present in PC12nnr5 cells under conditions in which TrkA was readily detected in normal PC12 cells. We then tested K252a, a specific TrkA inhibitor, for its ability to block p75NTR-mediated Akt phosphorylation using recombinant viruses encoding p75ICD and p75mICD, which are incapable of binding ligand. LacZ, p75ICD, and p75mICD adenoviruses were used at m.o.i. = 25 in these experiments since this infection level activated Akt (Fig. 2), but did not induce cell death (Fig. 1). Fig. 3B shows that K252a completely blocked NGF-mediated Akt phosphorylation in PC12 cells, but had no effect on p75ICD- and p75mICD-induced Akt phosphorylation in either PC12nnr5 or PC12 cells. Therefore, p75NTR-mediated activation of Akt occurs independently of TrkA signaling.

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p75NTR on Akt is that unliganded p75NTR functionally inactivates cellular sphingomyelinase, thereby reducing cellular ceramide levels and causing a consequent increase in PI3K activity, PIP$_3$ levels, and Akt phosphorylation. To address this, we compared the effects of p75NTR overexpression on Akt activation in normal and Niemann-Pick fibroblasts. Niemann-Pick fibroblasts are deficient in acidic sphingomyelinase, which is the only form of sphingomyelinase activated by p75NTR in PC12 cells. These data show that infection of PC12nnr5 cells with p75NTR and p75ICD adenovirus (all at m.o.i. = 25) and exposed to 0.5 μM staurosporine for 18 h, and levels of cellular apoptosis were determined by assessing annexin V binding by FACS analysis. Fig. 5A shows that infection of PC12nnr5 cells with p75NTR and p75ICD viruses significantly reduced the incidence of apoptosis compared with Me2SO-treated cells or cells infected with control LacZ virus.

To extend these results to other cellular models, TIMp75-3 cells were analyzed for p75NTR-mediated survival properties. TIMp75-3 cells were produced from the MG87 cell line, which undergoes rapid cell death in the absence of serum-derived growth factors. TIMp75-3 cells were treated with 2.5 μg of doxycycline for 36 h (to induce p75NTR expression) and then deprived of serum for 18 h. FACS analysis was performed to assess p75NTR expression and apoptosis. Fig. 5B clearly shows that TIMp75-3 population that expressed p75NTR was much less susceptible to cell death induced by serum deprivation, indicating that p75NTR promotes survival under these circumstances.

Activation of Akt by p75NTR Requires Active PI3K—To begin to determine the mechanisms used by p75NTR to activate Akt, the effect of PI3K inhibitors on p75NTR-induced Akt phosphorylation was examined. LY294002 and worthmann were first tested for their ability to block Akt phosphorylation induced by NGF in PC12 cells. Fig. 6A shows that, as expected, both inhibitors efficiently reduced NGF-induced Akt activation. Both inhibitors also completely blocked Akt phosphorylation induced by the p75mICD (Fig. 6B) or p75ICD or p75NTR (data not shown) recombinant adenovirus in PC12nnr5 cells and in A875 human melanoma cells. Therefore, PI3K activity is required for p75NTR-mediated activation of Akt.

Fig. 5. Expression of p75NTR facilitates cell survival. A, PC12nnr5 cells were infected with recombinant adenovirus encoding LacZ, p75ICD, or p75NTR at m.o.i. = 25; incubated for 24 h; and then treated with 0.5 μM staurosporine or vehicle (dimethyl sulfoxide (DMSO)) for 18 h and analyzed for annexin V binding. A minimum of 20,000 cells were analyzed for each condition shown. B, TIMp75-3 cells were treated with 2.5 μg/ml doxycycline (DOX) for 36 h and serum-deprived for 18 h. Flow cytometry was carried out to determine p75NTR expression levels (using antibody p75NTR-B1) and to determine the proportion of cells with sub-G$_1$ DNA content using propidium iodide staining. A minimum of 30,000 cells were analyzed by flow cytometry for each condition. Statistically significant differences determined by multiple analysis of variance are indicated with asterisks in A and B (*, p < 0.05; **, p < 0.001). Data in A and B represent the means ± S.E. of three independent experiments.

2 R. T. Dobrowsky, personal communication.
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DISCUSSION

We have demonstrated that p75NTR expression leads to Trk-independent, PI3K-dependent Akt phosphorylation. High levels of p75NTR expression increase JNK and c-Jun phosphorylation and promote apoptosis, yet lower p75NTR expression levels are associated with activation of Akt and suppression of apoptosis induced by distinct stressors. p75NTR expression levels that potentiate Akt phosphorylation and survival increase the phosphotyrosine content of several cellular proteins, including p85 and Shc, suggesting that p75NTR affects the activity of tyrosine kinases or PTPases. Consistent with this, we demonstrate that p75NTR expression is associated with a decrease in cytosolic PTPase activity.

Many studies have demonstrated that p75NTR can facilitate apoptosis. Our earlier work (46) has shown that overexpression of the p75NTR intracellular domain within neurons of transgenic mice results in dramatic loss of peripheral and central neurons, and Barde and co-workers (30, 55) has shown that embryonic retinal cells that express p75NTR under go cell death that can be prevented by the application of antibodies against either NGF or the p75NTR extracellular domain. Genetically altered mice rendered null at p75NTR or NGF loci show deficits in developmental apoptosis within the retina and spinal cord (31), and p75NTR can facilitate apoptosis of cultured rat oligodendrocytes (29) and sympathetic neurons (27). The precise pathways that p75NTR activates to induce apoptosis are unclear, but JNK, caspase activation, and increased p53 levels have been observed in some systems (26–28, 32). To reliably activate p75NTR signaling cascades, we created recombinant adenoviruses that encode either full-length p75NTR or the p75NTR intracellular domain. As expected from our earlier work in transgenic mice (46), adenovirus-mediated overexpression of p75ICD resulted in cellular apoptosis that was associated with increased JNK activity and c-Jun phosphorylation. Expression of p75NTR or myristoylated p75ICD gave similar results, with p75mICD proving a particularly potent apoptotic inducer. These reagents will be useful for studies designed to identify specific signaling events in the p75NTR apoptotic cascade.

Neurotrophin binding to p75NTR results in the activation of sphingomyelinase and the production of ceramide (20–22, 29, 56). Ceramide generated by p75NTR activation may inhibit Trk receptor activation (57), activate JNK (29, 58, 59), and affect neuronal differentiation (22). In some systems, sphingomyelinase activation results in a ceramide-dependent decrease in PI3P production and a subsequent reduction in Akt activity (42, 43), and our initial hypothesis was that p75NTR-dependent ceramide accumulation would suppress PI3K activity and thereby reduce Akt activation. However, overexpression of p75NTR resulted in ligand-independent activation of Akt in multiple cell types. Indeed, Akt was activated even at p75NTR expression levels that facilitate apoptosis, indicating that when the apoptotic pathway is activated, it can override the pro-survival effect of Akt.

The activation of Akt by p75NTR requires active PI3K and correlates with increases in the phosphotyrosine content of several proteins, including the adaptor protein Shc, the p85 regulatory subunit of PI3K, and a 120-kDa cell-surface protein. The increased phosphotyrosine content of these proteins correlates with reduced cytosolic PTPase activity in the presence of cytosolic PTPase activity, whereas membrane-associated PTPase activity was unchanged by p75NTR expression. These results indicate that p75NTR is capable of inhibiting cytosolic PTPase(s) and suggest that increases in the phosphotyrosine content mediated by p75NTR may be secondary to alterations in PTPase activity.
p75NTR-mediated inhibition of a cytosolic PTPase may therefore be a proximal event in the signaling cascade that may allow a Shc-PI3K complex to associate with the plasma-lemma and increase PIP₃ production. Additional studies will be required to identify specific phosphatase(s) inhibited by p75NTR, but one candidate is FAP, which physically interacts with p75NTR when overexpressed in 293T cells (38). FAP also binds human Fas (60), and Fas-mediated apoptosis can be suppressed by FAP (61, 62) through an unknown mechanism.

An alternative mechanism that may account for p75NTR-mediated Akt activation involves a link between TRAF proteins and Src kinases. The TRANCE receptor is a member of the TNFR superfamily that activates survival pathways in osteoclasts in part by activating Akt, and a recent study has found that a complex of Src kinase and TRAF-6 is required for this effect (63). p75NTR interacts with several members of the TRAF family, including TRAF-6 (25, 34), raising the possibility that this signaling path may also contribute to p75NTR-mediated Akt activation. Future experiments specifically examining FAP and Src signaling will be required to reveal the relative contributions of each of these pathways to p75NTR-mediated Akt activation.

The signaling mechanisms employed by p75NTR are not well understood, and the relationship of neurotrophin binding to p75NTR action remains unclear. All neurotrophins activate sphingomyelinase when bound to p75NTR (20, 21), but only NGF is capable of inducing apoptosis and nuclear factor-κB activation in most systems (23, 29, 30). Paradoxically, some studies suggest that the receptor signals apoptosis when free of ligand and that this function is suppressed by ligand binding to p75NTR (64, 65). For our studies, we produced a group of recombinant adenovirus that would constitutively activate p75NTR signaling and thereby allow us to identify p75NTR cascades irrespective of ligand binding. All of the p75NTR constructs employed specifically activate the JNK pathway and mediate apoptosis when expressed at high levels, indicating that they are capable of activating p75NTR signaling pathways. The high expression levels required to in-
duce apoptosis presumably reflect forced formation of a receptor signaling complex normally obtained in the presence of appropriate ligand (46). It is noteworthy that proximity of the intracellular domain to the plasma membrane appears important for activation of apoptosis by p75NTR since the p75ICD fragment elicits stronger apoptotic signaling than either p75NTR or p75ICD.

Cytotoxic effects are observed when p75NTR is highly overexpressed, but much lower expression levels of full-length p75NTR and the intracellular domain mutants elicit Akt phosphorylation and enhance survival. The canonical view of p75NTR action is that receptor signaling is activated by ligand binding, but recent studies on other TNFR superfamily members suggest an alternative paradigm for p75NTR signaling. Lenardo and co-workers (66, 67) have recently shown that some TNFR superfamily members must pre-assemble into cell-surface oligomers before binding ligand. Many investigators have observed oligomeric p75NTR in the absence of ligand in a variety of preparations (for an early example, see Ref. 54), consistent with the possibility that p75NTR may also pre-assemble into oligomers. With Fas and TNFR-1, ligand binding produces a conformational shift that enables specific signaling events, but does not alter the oligomeric nature of the receptor complex. Thus, the role of ligand is to shift the pre-assembled receptor complex to a different signaling mode. All three p75NTR constructs used in our studies elicited Akt activation when produced at low expression levels, but much higher levels of expression were required for apoptotic signaling. We favor the hypothesis that these signaling events reflect distinct p75NTR signaling complexes; in the ligand-free state, p75NTR will constitutively activate signaling pathways that involve Akt, and when bound by ligand, it activates signaling pathways that result in cellular apoptosis.

In conclusion, we have identified a novel p75NTR signaling pathway that results in phosphorylation of Akt and that enhances cell survival. These data suggest that the autonomous signaling role of p75NTR may be broader than previously considered, with p75NTR capable of signaling pathways that support survival and death under different cellular circumstances.

Acknowledgments—We are grateful to Farid Arab Said and Sandra MacPherson for technical assistance with adenoviruses; Alain Boud-
The p75 Neurotrophin Receptor Activates Akt (Protein Kinase B) through a Phosphatidylinositol 3-Kinase-dependent Pathway
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J. Biol. Chem. 2001, 276:23097-23104.
doi: 10.1074/jbc.M011520200 originally published online April 18, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M011520200

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