The cytoplasmic juxtamembrane region of the p75 neurotrophin receptor (p75NTR) has been found to be necessary and sufficient to initiate neural cell death. The region was named “Chopper” to distinguish it from CD95-like death domains. A 29-amino acid peptide corresponding to the Chopper region induced caspase- and calpain-mediated death in a variety of neural and non-neural cell types and was not inhibited by signaling through Trk (unlike killing by full-length p75NTR). Chopper triggered cell death only when bound to the plasma membrane by a lipid anchor, whereas non-anchored Chopper acted in a dominant-negative manner, blocking p75NTR-mediated death both in vitro and in vivo. Removal of the ectodomain of p75NTR increased the potency of Chopper activity, suggesting that it regulates the association of Chopper with downstream signaling proteins.

The p75 neurotrophin receptor (p75NTR), a member of the tumor necrosis factor receptor (TNFR) family, was the first receptor to be described for nerve growth factor (NGF) and can bind to each of the neurotrophins (brain-derived neurotrophic factor, neurotrophin-3, and neurotrophin-4/5) with similar equilibrium constants (2, 3). Subsequently, the Trk (tyrosine kinase receptor) family, members of which have varying specificity for each neurotrophin, was described (4). Ligand binding to a Trk receptor activates a phosphorylation signal cascade, resulting in cell survival and process outgrowth (4, 5); and p75NTR has been shown to facilitate Trk signal transduction by the formation of high affinity neurotrophin receptor complexes (6).

p75NTR can mediate cellular effects independently of association with Trk, such as the activation of nuclear factor-κB (7–10), c-Jun (10, 11), and ceramide (12, 13). Although providing a definite function for p75NTR signaling remains controversial, there is substantial evidence to support the hypothesis that p75NTR can initiate a neural cell death pathway. Overexpression of p75NTR increases cell death in Trk-expressing cells (14–17), whereas lowered expression of p75NTR decreases neuronal death both in vitro and in vivo (11, 18–20). Furthermore, the apparent activation of p75NTR by neurotrophins in cells lacking the appropriate Trk receptor results in cell death in a number of experimental paradigms. The addition of NGF to developing chick optic nuclei or NGF (attached to glass beads) applied to developing chick retinas leads to increased cell death (21, 22), whereas the addition of blocking antibodies to either NGF or p75NTR decreases naturally occurring chick retinal cell death (23). Cultured sympathetic neurons and proopioergic neurons also display increased cell death after the addition of brain-derived neurotrophic factor and NGF, respectively; and it was again possible to block this cell death by application of p75NTR antibodies (11, 24). Oligodendrocytes and Schwann cells also show p75NTR-dependent cell death after treatment with NGF, provided TrkA is not coexpressed (8, 25).

p75NTR has sequence similarity to other TNFR family members both in the cysteine-rich ectodomain and in the cytoplasmic sequence known as a “death domain” (26). Despite the presence of a death domain in p75NTR, there is accumulating evidence that this region does not mediate the ability of p75NTR to promote cell death. Unlike the TNFR death domain, the death domain of p75NTR does not interact with other death domain-containing proteins (16), does not spontaneously multimerize in solution (27), and does not function in the same manner (28). Moreover, we have recently shown that without the juxtamembrane sequence, p75NTR is unable to induce neuronal cell death, whereas deletion of the death domain sequence has no effect on the ability of p75NTR to kill (16).

Little is known about the downstream components of the p75NTR death signaling pathway, although it is known that caspases are ultimately activated (see Ref. 29). Bcl-2 does not inhibit cell death induced by p75NTR (16, 25) and can in fact promote p75NTR-mediated cell death in some circumstances (16). In contrast, Bcl-xL can inhibit p75NTR-mediated cell death (16). Recently, the six TRAF proteins, which are capable of activating nuclear factor-κB, have been shown to associate with various regions of the p75NTR cytoplasmic domain (30, 31). Two novel zinc finger proteins, neurotrophin receptor interacting factor and SC-1, have also been found to interact with the cytoplasmic domains of p75NTR and appear to mediate cell death (32, 33). However, the precise role of these proteins in the p75NTR death signaling is unclear at this stage. Further characterization of the responsible death-inducing portion of p75NTR may ultimately help to determine the role of these and other p75NTR-interacting proteins in p75NTR and Trk signaling.

To determine how the p75NTR death signal is transduced, the components within the p75NTR cytoplasmic region required for death signal initiation were sought. The domain that is both...
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necessary and sufficient for initiating p75<sub>NTR</sub> cell death was found to be the cytoplasmic juxtamembrane 29-amino acid sequence we have named “Chopper.” It was found that membrane attachment was critical for cell death initiated by Chopper and that the ectodomain regulated Chopper activity. Chopper was shown to kill via a calpain- and caspase-mediated death pathway that is independent of Trk signaling and that is present in a variety of cell types.

**MATERIALS AND METHODS**

**Cell Culture**—Dorsal root ganglia were dissected from postnatal day 0 C57Bl/6 mice and cultured as described previously (16). PC12 cells were grown in Dulbecco’s modified Eagle medium containing 10% fetal bovine serum and 5% horse serum (Commonwealth Serum Laboratories), differentiated with NGF (50 ng/ml; Peprotech) and serum withdrawal, and cultured for a further 5 days prior to microinjection. Wild-type Schwann cells and the 293T and M1 cell lines were maintained in Dulbecco’s modified Eagle’s medium and 10% fetal bovine serum.

**Microinjection and Transfection**—Sensory dorsal root ganglion neurons, rat PC12 cells, and human 293T fibroblast cells were microinjected with DNA at individual concentrations ranging from 100 to 20 μg/ml as described previously (16). Unless otherwise stated, DNA concentrations were 100 μg/ml. Where two plasmids were injected together, the total DNA concentration did not exceed 220 μg/ml, and toxicity was not observed at this concentration. Live cells were identified by their phase-bright morphology and, in some cases, propidium iodide exclusion.

Transient transfections of PC12, 293T, and wild-type Schwann cells were performed using Effectene (QIAGEN Inc.), and the numbers of live GFP-expressing cells were determined by FACS or cell counts. Cell survival of sptc35-transfected cells was compared with cells expressing GFP vectors. M1 cells were treated with 2 μM Palm29p, and cell survival was compared with PalmCpen-treated cells. GFP protein expression levels were determined 24 h after transfection by FACS. Schwann cells, stably transfected with Bcl-2 (25), were transfected with GFP or c35 by a CaCl<sub>2</sub> method and glycerol shock (34) and then sorted together, the total DNA concentration did not exceed 220 μg/ml, and toxicity was not observed at this concentration. Live cells were identified by their phase-bright morphology and, in some cases, propidium iodide exclusion.

**RESULTS**

**p75<sub>NTR</sub> Mediates Cell Death through the Cytoplasmic Juxtamembrane Domain Called Chopper**—To fully characterize the critical region of the p75<sub>NTR</sub> cytoplasmic domain that is both sufficient and necessary to mediate cell death, a series of expression constructs were made in which one or more protein domains of rat p75<sub>NTR</sub> were deleted (Fig. 1A). The ability of the resulting truncated p75<sub>NTR</sub> proteins to mediate cell death was determined by microinjection of these constructs into the nuclei of freshly isolated sensory neurons cultured in the presence of leukemia inhibitory factor (LIF) and measuring cell survival over the next 24 h.

Constructs retaining the cytoplasmic juxtamembrane 35-amino acid domain along with the ectodomain (p75<sub>NTR</sub>, p75<sub>NTR</sub>Δ<sub>cyt</sub>) were able to mediate neuronal death, whereas those lacking this domain (p75<sub>NTR</sub>Δ<sub>ect</sub>, p75<sub>NTR</sub>Δ<sub>ect-cyt</sub>) were not (Fig. 1B), a result that is in agreement with earlier findings (16). This indicated that the domain required for p75<sub>NTR</sub> death signaling is the juxtamembrane domain and not the region with homology to the CD95 and TNFR death domains.

To determine whether the ectodomain of p75<sub>NTR</sub> is necessary to mediate the death signaling pathway, a construct lacking the ectodomain (but retaining the signal peptide sequence, transmembrane domain, and entire 152 amino acid cytoplasmic domain) p75<sub>NTR</sub>Δ<sub>cyt</sub> was tested for its ability to kill. It was found that removal of the ectodomain significantly increased the killing ability and more than doubled the amount of cell death induced by full-length p75<sub>NTR</sub> (Fig. 1B). The construct was then further truncated, removing the region with homology to the CD95 and TNFR death domains.

Oligonucleotide sequences are available on request.
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Free Chopper Inhibits p75NTR-mediated Killing—As c35 was unable to induce cell death, we examined whether it might act to inhibit p75NTR-mediated cell death by a dominant-negative mechanism. When c35 was coexpressed with p75NTR, it was found to inhibit cell death in a dose-dependent fashion (Fig. 2A).

To ascertain whether the Chopper sequence also mediates cell death in other paradigms of p75NTR-mediated death, we tested the effect of c35 expression in a Schwann cell system where it has been shown that NGF induces cell death through endogenous p75NTR (25). Transfected Schwann cells expressing c35 or GFP were plated in the presence or absence of NGF, and their survival was monitored over a 24-h period. c35 expression was found to significantly abrogate Schwann cell death, blocking ＞90% of NGF-induced cell death compared with the GFP control (Fig. 2B). This strongly suggests that NGF-induced Schwann cell death is mediated through a Chopper-dependent pathway.

Previously, we had shown that Bcl-2 enhanced neuronal death mediated by p75NTR overexpression (16), so the effect of c35 on this neuronal death pathway was also examined. When c35 was coexpressed with p75NTR and Bcl-2, it again totally abrogated the effects of p75NTR-mediated killing (Fig. 2C), again suggesting that this pathway is dependent on Chopper activity.

The ability of c35 to inhibit neuronal cell death due to stress stimuli, namely NGF withdrawal, was also tested. No increase in survival of neurons treated with c35 was observed after 48 h (Fig. 2D). This result was consistent with our previous observation that the death pathway used by cells dying from stress stimuli is distinct from the p75NTR-mediated death pathway (16).

Free Chopper Peptides Prevent Death Signaling in Vitro and in Vivo—To further examine the requirements for death induction by the Chopper domain and to confirm the inhibitory action of c35, cell-permeable p75NTR-derived peptides were synthesized. Due to ease of synthesis, a 29-residue (lacking the 6 carboxyl-terminal amino acids), in addition to the 35-residue peptide of the Chopper sequence, was synthesized and modified as shown in Fig. 3A (see “Materials and Methods”). To facilitate intracellular delivery of synthetic p75NTR-derived peptides, a 17-residue cell-permeable penetratin peptide (35) was conjugated to p75NTR peptides via a disulfide bond (see “Materials and Methods”). This linkage is stable in the extracellular, but is rapidly reduced once the complex is internalized, liberating the p75NTR peptide from penetratin (37).

Fluorescin-labeled p75NTR-derived c29 peptide conjugated to penetratin (c29pen) was rapidly taken up by neurons and was observed throughout the cell, as judged by fluorescence microscopy. Moreover, using FACS, we found that c29pen was quickly internalized by both control and c35-expressing cells, but this process was inhibited by c35 (Fig. 3B).

To examine the effect of p75NTR peptides on cell death signaling, we transfected 293T cells with GFP from an IRES-EGFP expression construct and various p75NTR expression constructs. Expression levels did not significantly differ between constructs expressing truncated p75NTR forms and full-length p75NTR-expressing vector (Fig. 1C). The results indicated that the membrane-linked 35-amino acid domain was required not only for p75NTR-mediated death signaling, but was, by itself, sufficient to induce cell death that was of greater potency than when it was linked to other domains. This killing domain of p75NTR has been named Chopper to distinguish it from the CD95 homologous death domain region.

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microscopy including confocal imaging (Figs. 3B and 4, A, C, and F). At a peptide concentration of 10 or 15 μM, all neurons were found to be fluorescent after 30 min, whereas at 3 μM, <20% of the cells were fluorescent at this time point (Fig. 3B). Longer treatment of cells with 3 μM peptide (2 h) did, however, result in all cells taking up the peptide (data not shown). In contrast, no appreciable uptake of non-conjugated peptides (c29) could be detected (Fig. 4, B, D, and F), even after a 24-h incubation with 15 μM peptide (data not shown). Treatment for up to 2 h with a 15 μM concentration of the penetratin-linked free Chopper peptides had no significant effect on neuronal survival after 16 h in either NGF (76.2 ± 7.9%) or LIF (88.3 ± 3%) compared with untreated cells (86.6 ± 1.8 and 85.4 ± 5.3%, respectively); however, toxic effects were observed when neurons were treated with penetratin alone or penetratin-linked peptides at concentrations >20 μM and applied for >2 h (data not shown).

To test the ability of these p75NTR peptides to prevent cell death, neurons were injected with sptc35 or control GFP expression plasmids and then treated for 30 min with 15 μM synthetic c35-penetratin conjugate (c35pen) or penetratin alone (pen). Neurons injected with sptc35 and then treated with cell-permeable c35pen peptide had a significant survival advantage over penetratin-alone treated cells (Fig. 5A). The 29-residue peptide (c29pen) lacking the 6 carboxyl-terminal residues of c35 was found to confer equivalent protection against sptc35-mediated cell death (Fig. 5A).

The free Chopper peptides were then utilized to determine whether the Chopper domain functions during development of the nervous system. Developmental cell death of chick retinal ganglionic neurons occurs during early embryogenesis (E3–7) and has been shown to be mediated through NGF and p75NTR (22, 23). To test if Chopper could inhibit retinal ganglionic cell death, various peptides (pen, c29pen, and c29) were injected.
into the vitreous of E4.5 chick eyes in ovo. At E5.5, the retinal ganglial layer was dissected from the embryos and analyzed for apoptotic cell death. c29pen was found to significantly reduce the degree of cell death by 40% (Fig. 5B), indicating that the Chopper domain is involved in initiating downstream signaling of the p75NTR cell death pathway during the period of naturally occurring cell death.

Lipid-modified Chopper Peptides Cause Cell Death—Our observations that transmembrane Chopper (sptc35) was a potent inducer of death and that free Chopper specifically inhibited this activity suggested that both proteins bound to the same accessory protein(s), but that membrane localization was required to activate the death pathway. To further explore whether the killing activity of p75NTR requires the transmembrane sequence or whether membrane attachment per se is sufficient, lipid-modified (palmitoylated) forms of pro-survival Chopper peptides were generated to localize them to the plasma membrane (38, 39). The synthetic 29-mer Chopper peptide was modified by conjugation of lysine-ε-palmitoyl and fluorescein moieties to the amino terminus (Palm29pen) (Fig. 3A); and its cellular localization and ability to induce neuronal death, when delivered as a penetratin conjugate, were examined.

Interestingly, the addition of the lipid group reduced the concentration of penetratin-linked peptide required for cellular uptake: $97 \pm 4.2\%$ of the cells were visibly fluorescent after incubation in 2 μM Palm29pen for 30 min compared with <20% with c29pen treatment (see Fig. 3B). In contrast to cells treated with non-palmitoylated peptides (Fig. 4, A, C, and E), cells often associated with membranes and was especially obvious in membranes that had begun to form large blebs at the surface of neurons (G, arrows). Other neurons showed accumulation of fluorescence in vacuoles that appeared to be budding off at the cell surface (H). By 2 h, many of the neurons that had taken up the palmitoylated c29pen peptide (J) were dead, as shown by their crenated and shrunken appearance (I) and the uptake of propidium iodide into their nuclei (K). A, B, and J, phase-contrast micrographs (bar = 50 μm); C, D, and J, conventional fluorescence micrographs (bar = 50 μm); E–H, single confocal fluorescence micrographs (bar = 5 μm).
treated with Palm29pen showed visible changes in morphology within 1 h of treatment: the neurons had large surface vacuoles or “blebbing” (Fig. 4, G and H) reminiscent of classical apoptosis (40, 41). The localization of palmitoylated Chopper was throughout the cell, but was particularly noticeable in the plasma membrane of the vacuoles (Fig. 4G); and fluorescein was highly concentrated in membrane “blebs” attached to the cell body and neurites (Fig. 4H). In addition, dead cells had morphological changes consistent with apoptosis (41), including creased appearance and condensed, propidium iodide-positive chromatin (Fig. 4, I, J, and K). Indeed, neurons treated with the palmitoylated Chopper peptide underwent very rapid cell death within the 2-h treatment period, in contrast to cells treated with non-palmitoylated peptides: an unrelated peptide, PalmCpen (derived from gp130), that was also palmitoylated and conjugated to penetratin (Fig. 6A), or Palm29, which was not conjugated to penetratin (data not shown), none of which showed evidence of morphological changes. This suggested that Chopper initiated a death signal shortly after peptide internalization, but only when it interacted with the plasma membrane. Moreover, it strongly suggested that the transmembrane sequence of p75NTR (85–293) is a necessary component of p75NTR activation, present in dorsal root ganglial neurons, is lacking in 293T cells.

Recently, the PEST domain of IκB was shown to bind to the calmodulin-like domain of the large subunit of the μ-calpain protease, and such interaction, in the presence of calcium, was sufficient to activate calpain (46). The presence of a PEST protease, and such interaction, in the presence of calcium, was sufficient to activate calpain (46). The presence of a PEST sequence was present in a variety of cell types and was independent of the presence of Trk. Although sptc35 was a potent inducer of death in 293T cells (29 ± 1%), full-length p75NTR and p75NTR85 (85 ± 2.6%) surprisingly did not induce any 293T cell death, even in the presence of 50 ng/ml NGF, suggesting that a component of p75NTR activation, present in dorsal root ganglial neurons, is lacking in 293T cells.

The Juxtamembrane Domain of p75NTR Mediates Cell Death

Since p75NTR has been shown to be normally palmitoylated at cysteine 279, which is within the Chopper domain (42), we examined whether this residue may be critical to killing. Because Palm29pen was palmitoylated through a modified lysine residue and because the peptide might be further palmitoylated at cysteine 279 (used for penetratin conjugation) after reduction of the disulfide bond (Fig. 3A), cysteine 279 was substituted in the sptc35 expression construct with alanine. Unlike sptc35, expression of sptc35 C→A in neurons did not induce cell death (Fig. 6B), indicating that Cys279 is necessary for cell killing activity of Chopper.

Chopper Death Signaling Is Independent of Trk and Is Mediated through Calpains and Caspases—The ability of palmitoylated Chopper peptides to kill within 2 h of application suggested that the downstream components of the death pathway were already present in the neurons and did not require up-regulated expression for death induction. To determine whether these components are found specifically in neurons, the ability of membrane-associated Chopper (expression constructs and peptides) to kill a range of cell types was determined. Membrane-associated Chopper was found to kill Schwann cells, 293T fibroblast cells, and M1 lymphocytes, which do not express Trk (43–45), as well as sensory neurons and differentiated and undifferentiated neuroblastoma PC12 cells (see “Materials and Methods”; data not shown). This implied that the death machinery utilized by the Chopper sequence was present in a variety of cell types and was independent of the presence of Trk. Although sptc35 was a potent inducer of death in 293T cells (29 ± 1%), cell survival was significantly lower from c35-expressing neurons (standardized to 100%) (mean ± S.E., n = 2). *** p < 0.001.

Caspases have previously been shown to be a necessary component of the p75NTR death pathway (16, 25, 48). To ascertain whether caspases as well as calpains are a component of the Chopper death pathway, cell-permeable peptide inhibitors of caspases were employed (49). Neurons were treated with t-butoxycarbonyl-D-fmk (Boc-D-fmk) and benzoyloxyacarbonyl-DEVD-fmk (zDEVD-fmk) (B). The number of live cells, grown in NGF, was counted prior to peptide treatment (t = 0), and then cell death was determined 17 h after removal of peptides. The graphs have been standardized to show c29pen cell death as 0% (mean ± S.E., n = 3).
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DISCUSSION

A New p75NTR Death Domain: Chopper—The study has identified a juxtamembrane 29-amino acid region of p75NTR as being both required for and sufficient to induce rapid cell death. This region, along with the transmembrane region, is the most highly conserved sequence of p75NTR, 95% identity between rat, human, and chicken (47). It is not, however, conserved between other members of the TNFR/CD95 family of death receptors and shows little sequence homology to other known protein domains. The region does, however, contain a PEST sequence (residues 287–300), a region rich in proline, aspartate, glutamate, serine, and threonine residues flanked by clusters rich in basic amino acids, which has traditionally been associated with accelerated protein degradation through facilitating interactions with other molecules (see below for further discussion) (51). This new juxtamembrane death-promoting region of p75NTR has been named Chopper to distinguish it from the putative death domain previously ascribed to p75NTR on the basis of its homology to the death-activating domains, especially type II found in the TNFR/CD95 receptor family (26). Our finding confirms work demonstrating that p75NTR does not contain a domain that functions in the same manner as the Fas receptor death domain (16, 28). The inhibition of p75NTR-mediated developmental cell death in the chick retina by free Chopper peptides was to a similar extent as that previously demonstrated with anti-NGF or anti-p75NTR antibodies (23) and further supports the idea that the Chopper domain is involved in initiating downstream death signaling. There remains the possibility, however, that motifs within the death domain may contribute to death signaling in particular circumstances (52, 53).

Downstream Signaling through Chopper—Chopper was found to initiate cell death only if attached to the plasma membrane through its transmembrane region or if palmitoylated. In addition to targeting Chopper to the membrane, palmitoylation of p75NTR may play a more direct role in activation since mutation of cysteine 279 to alanine, through which p75NTR is normally palmitoylated (42), results in loss of killing function, despite Chopper being localized to the membrane via the transmembrane domain. Palmitoylation is a reversible post-translational modification, targeting proteins to subregions of the plasma membrane known as lipid rafts (54, 55). For instance, palmitoylation is required for Ras to localize to lipid rafts, where it interacts with other membrane-localized signaling proteins to initiate signal transduction pathways (56). Similarly, lipid modification is required for the receptor clustering function of the Rapsyn protein (57). By increasing the local concentration of Chopper and accessory protein complexes, palmitoylation may, like multimerization of Fas and TNFR (58, 59), lead to initiation of death signaling. Palmitoylation has also been shown to promote high affinity interactions (39) that may favor the association of the Chopper domain with binding partners.

The ability of non-membrane-bound Chopper to inhibit in a variety of p75NTR-mediated neural death models including developmental cell death in vivo implies a dominant-negative mechanism whereby both the membrane-bound form and the free form interact with the same accessory protein(s). Possible unidentified interacting partners include proteins that could be localized to the same lipid microdomains as palmitoylated p75NTR (39, 60, 61). Free Chopper peptides may prevent cell death by sequestering such proteins in the cytoplasm and thus away from membrane-associated Chopper.

Candidates for the role of Chopper-interacting molecules are members of the TRAF family, which have been shown to mediate both cell survival and death signaling in non-neural cells (62, 63). The TRAF proteins are also implicated in NGF signaling through p75NTR (7, 30, 64, 65). Co-immunoprecipitation studies have indicated that all six TRAF proteins are associated with, but may not directly bind to, p75NTR (16, 30, 31) and that the association is dependent on neurotrophin binding in some cases (30, 31). Both TRAF4 and TRAF6 have been shown to associate with the juxtamembrane region, whereas TRAF2 bound to the carboxyl-terminal region. Closer examination using deletion mutants showed that residues 300–315 of the juxtamembrane region of p75NTR were required for the interaction with TRAF6 to occur (30). Since only 3 amino residues of this domain overlap with the Chopper domain, TRAF6 is unlikely to be involved in downstream signaling.

Two zinc finger proteins, neurotrophin receptor interacting factor (32) and SC-1 (33), have recently been shown to directly interact with the juxtamembrane domain of p75NTR, and both have been shown to translocate to the nucleus after NGF binding to p75NTR (32, 33). Although little is known about the gene targets of these transcription factors, mice with a targeted deletion in neurotrophin receptor interacting factor have been shown to have reduced levels of neuronal death in the developing retina, suggesting that it plays a role in mediating neuronal death (32) and thus may be downstream of Chopper.

Evidence herein suggested that calpain participates in the p75NTR death pathway possibly by interacting directly or indirectly with the PEST domain within Chopper. Calpains are a family of calcium-activated cysteins/thiol transferases that are highly expressed in brain and that play a role in mediating neuronal apoptosis and necrosis in several chronic neurodegenerative diseases, in neural injury, and after ischemia (66). Calpain, in some cases, has been shown to be more important than caspases in mediating in vitro models of neuronal death (66, 67). Although their substrate recognition sequence is different, activated calpains and caspases cleave a number of common proteins important in apoptotic pathways and can also cleave each other (66, 67). Thus, it is possible to inhibit calpain-initiated death signaling with traditional blockers of caspases such as DEVD, which inhibits caspase-2, -3, and -7 (50, 67), which we have shown to abrogate the ability of Chopper to kill (this study and Refs. 16, 25, and 48). Therefore, both calpain and caspases may be p75NTR-mediated cell death effectors within the same signaling pathway.

It has recently been shown that p75NTR can regulate cellular calcium levels, causing an influx of calcium into the cell after NGF stimulation (68), suggesting that p75NTR may interact with an ion channel or channel regulator. Furthermore, many studies show that membrane localization of calpain is necessary for its activation (66), which would explain the requirement for membrane attachment for Chopper killing action. These data are in agreement with our model whereby activation of p75NTR/Chopper leads to calcium influx, activation of calpain, and rapid cell death. In addition, calpain-mediated cell death is not prevented by overexpression of Bcl-2 (67); and thus, calpain-mediated cell death is consistent with our observation that Bcl-2 does not inhibit p75NTR-mediated cell death and can, in some cases, promote this death (16, 25).

Activation of Chopper—One of the surprising findings was that Chopper death signaling was substantially inhibited by the presence of the ectodomain of p75NTR and that, in 293T cells, there was no observable killing unless the ectodomain was removed. The ability of NGF to override the death signal in Trk-expressing cells only when p75NTR retained the ectodomain highlighted this and suggested that the ectodomain regulates Chopper activity by controlling receptor clustering or perhaps by regulating calcium influx. This raised the question...
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of whether removal of the p75NTR ectodomain could be the cause of physiological activation.

A wide variety of cell-surface proteins (69–71), including p75NTR (72–75), are released from the plasma membrane by extracellular cleavage. Metalloproteases, which cleave at a specific distance from the membrane rather than at sequence-specific sites, have been shown to be responsible in most cases (76, 77). p75NTR cleavage is indeed inhibited by metalloprotease inhibitors (75), and cleavage also correlates developmentally and temporally with p75NTR acting as a death inducer, e.g. after sciatic nerve lesion (72, 75, 78, 79). Therefore, we propose that physiological activation of the p75NTR death pathway might be a physiological mechanism for activating the cell killing ability of p75NTR. Cleavage of the ectodomain would leave a truncated protein in the membrane equivalent to the protein produced by the sptc152 construct, which we have shown to be far more effective at inducing cell death than full-length p75NTR. Activation of p75NTR death signaling by removal of the ectodomain may explain the perceived paradoxical circumstances where p75NTR induces death because a variety of environmental and developmental conditions, such as nerve lesion or spinal cord injury, may cause a variety of environmental and developmental conditions, which we have shown to be far more effective at inducing cell death than full-length p75NTR. Activation of p75NTR death signaling by removal of the ectodomain may explain the perceived paradoxical circumstances where p75NTR induces death because it causes physiological activation.

Conclusion—A truncated protein containing the juxtamembrane 29-amino acid p75NTR sequence was shown to be capable of inducing cell death when linked to the plasma membrane and was active in a number of cell death paradigms. Evidence was provided that the death pathway, which did not require Trk signaling, was likely to be activated by binding of accessory proteins (present in a variety of cell types) to the juxtamembrane domain, resulting in caspase and calpain activation. It is proposed that physiological activation of the p75NTR death pathway might be due to ectodomain cleavage of p75NTR, releasing a soluble ectodomain and yielding a death-inducing, transmembrane-linked form of p75NTR.

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The Juxtamembrane Domain of p75NTR Mediates Cell Death

Chopper, a New Death Domain of the p75 Neurotrophin Receptor That Mediates Rapid Neuronal Cell Death
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doi: 10.1074/jbc.M005214200 originally published online July 5, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M005214200

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