INTRODUCTION

Neurotrophins are target-derived soluble factors required for neuronal survival. Nerve growth factor (NGF) and basic fibroblast growth factor (bFGF) are two proteins that act as pro-survival factors for primary sensory and sympathetic neurons. However, several studies have suggested that NGF may also act as an apoptosis-inducing agent (Greene and Kaplan, 1995). The p75 neurotrophin receptor is the founding member of the TNF receptor superfamily, which includes the Trk family of receptor tyrosine kinases. The p75 receptor binds to NGF, BDNF, NT-3, and NT-4, and its expression is regulated by the levels of trophic factors. The p75 receptor has been shown to be involved in the regulation of cell survival and differentiation, and its expression is upregulated in response to axotomy. The study of the p75 receptor has been facilitated by the development of monoclonal antibodies specific for an epitope on the rat p75 receptor (Kaplan et al., 1992). These antibodies have been used to investigate the role of the p75 receptor in apoptosis, and have revealed that the p75 receptor may act as an apoptosis-inducing agent.

Previously, we have reported that the injection of NGF in newborn rats resulted in a decrease in the number of viable cells in the superior cervical ganglion of embryonic day 16 and newborn rats (Johnson et al., 1994). Although this report has not been widely acknowledged, the implication of these findings is that NGF may act as an apoptosis-inducing agent.

A more direct role for NGF in neuronal apoptosis was suggested by the injection of NGF in newborn rats that had received a facial nerve transection. This treatment resulted in a 50% reduction in cell number (Sendtner et al., 1992). Notably, these motor neurons upregulated the levels of p75 upon axotomy (Raivich and Kreutzberg, 1987; Yan and Johnson, 1988). Similarly, administration of NGF in chicks produced developmental neuronal cell death of the avian isthmo-optic nucleus, which expressed high levels of p75 and TrkB, but not its cognate receptor, TrkA (von Bartheld et al., 1994). Several of these observations may be explained by inappropriate competition of neurotrophins for their receptors. A newer and more interesting mechanism is that p75 may generate intracellular signals for inducing apoptosis selectively in certain cell contexts.

Here we review recent findings on the role of p75-dependent cell death during development and during injury conditions. Despite much current interest in this topic, the mechanisms by which neurotrophins regulate cell survival remain largely unknown. The role of p75 in neuronal apoptosis is an important area of research, and further studies are needed to elucidate the mechanisms by which neurotrophins promote or inhibit cell death.

KEY WORDS p75 receptor; TNF receptor superfamily; cysteine motif; ligand binding domain

Received 14 December 1998; accepted in revised form 28 December 1998
Fig. 1.

Fig. 2.
p75 AS A POSITIVE REGULATOR OF CELL SURVIVAL AND DIFFERENTIATION

In neurotrophin-responsive neuronal populations, p75 and Trk receptor members are frequently co-expressed, particularly in the vertebrate peripheral nervous system. The p75 receptor provides a positive modulatory influence on TrkA function (Barker and Shooter, 1994; Horton et al., 1997; Verdi et al., 1994), by increasing the number of high-affinity binding sites (Mahadeo et al., 1994). Responsiveness to low concentrations of NGF and the ability to form high-affinity sites are dependent on the relative levels of p75 and TrkA receptors (Benedetti et al., 1993; Verdi et al., 1994). Regulation of high-affinity site formation by coexpression of trkA and p75 provides an explanation for how these receptors may cooperate to increase neurotrophin responsiveness during development. Indeed, neuronal cell lines express both TrkA and p75 receptors and TrkA autophosphorylation is enhanced, leading to a faster differentiative response with NGF, as assayed by more rapid growth arrest and neuronal maturation (Verdi et al., 1994).

Cell survival is enhanced by a higher ratio of p75 to Trk receptors. This model has been supported by studies of the p75 null mice (see Table 1). In the absence of p75, there are selective losses in sensory and sympathetic innervation (Davies et al., 1993; Lee et al., 1992, 1994) consistent with, but not as severe as phenotypes exhibited by NGF, BDNF, NT-3 and TrkA, TrkB, and TrkC null mice (Snider, 1994). Sensory neurons from p75-/- embryonic sensory trigeminal neurons and neonatal sympathetic neurons also show a nearly fourfold greater requirement for NGF for cell survival compared to wild type neurons (Davies et al., 1993; Lee et al., 1994). Although these neurons are fully capable of responding through the TrkA receptor in the absence of p75, they do so with a significantly lower sensitivity.

Sympathetic neurons from neonatal p75-null mice required higher concentrations of NGF to survive than neurons from normal mice at earlier developmental stages (Lee et al., 1994). Two models have been proposed to account for how p75 can modulate Trk receptor function. The first is a ligand passing mechanism, which predicts that the high-affinity state is the
result of ligand presentation by p75 to the TrkA receptor (Barker and Shooter, 1994). The second model predicts that p75 and TrkA are capable of a ligand-independent association, which produces a high-affinity binding interaction (Chao and Hempstead, 1995). Such a mechanism would predict that conformational changes in the receptors would occur to facilitate ligand binding. This idea is supported by ligand-independent clustering and interactions between the two receptors observed in co-patching and biophysical measurements (Ross et al., 1996). All of these observations are consistent with the hypothesis that p75 can serve as a positive influence upon TrkA function.

**p75 AS A CONSTITUTIVELY ACTIVE PRO-APOPTOTIC RECEPTOR**

The hypothesis that p75 acts as a death receptor was realized by Bredesen and his colleagues from experiments conducted in cultured cell lines (Rabizadeh et al., 1993). Based on the observation that immortalized neural cells overexpressing p75 display an enhanced rate of apoptosis in response to serum withdrawal, a mechanism of ligand-independent death was proposed. According to this model, apoptosis promoted by p75 can be negated after binding to NGF. How NGF gives a survival signal through binding to p75 has not been established. However, the correlation between high levels of p75 expression and susceptibility to apoptosis following growth factor withdrawal in PC12 cells supports this mechanism of cell death (Barrett and Georgiou, 1996). Furthermore, down-regulation of p75 expression in neonatal dorsal root sensory neurons, using an antisense strategy, reveals enhanced survival (Barrett and Bartlett, 1994). Table 2 lists examples of ligand-independent apoptosis induced by p75 expression.

The identification of a 70–80 amino acid motif in the p75 intracellular sequence analogous to the death domain of the Fas and TNF receptor, p55, further consolidated the possibility of a cell death function for p75, similar to other family members (Feinstein et al., 1995; Hofmann and Tschopp, 1995). The sequence comparison was further supported by the NMR structure of the cytoplasmic domain of p75 (Liepinsh, 1997), which showed a striking similarity in overall three-dimensional structure to the Fas receptor death domain (Huang et al., 1996) and the death effector domain of the Fas-associated death domain protein, FADD (Eberstadt et al., 1998).

The phenotype of the p75 null mice not only supports a role in neuronal survival (Davies et al., 1993; Lee et al., 1994a,b) but an apoptotic function as well. Analysis of mice has revealed a significantly higher number of cholinergic neurons in the basal forebrain in p75-/- mice compared to wild type controls (Van der Zee et al., 1996; Yeo et al., 1997). These observations indicate that the absence of p75 results in enhanced survival, similar to the antisense effects observed in postnatal sensory neurons (Barrett and Bartlett, 1994). Expression of p75 in cholinergic neurons appears to result in an apoptotic outcome in this instance. This result has been contested by other stereological studies that argue that the number of neurons does not differ between wild type and p75-deficient animals (Peterson et al., 1997). A potential explanation to reconcile these opposing conclusions may lie in the genetic backgrounds of the mice that were examined.

An alternative explanation is the recent finding of an alternatively spliced isoform of the p75 receptor, lacking exon 3, which encodes the second, third, and fourth cysteine repeats in the extracellular domain of the receptor (Dechant and Barde, 1997). This region has been identified as the neurotrophin-binding domain. This transcript in the p75-mice would produce a receptor that contains the transmembrane and intracellular domains of p75, without the NGF-binding domain. It has been established that overexpression of the intracellular domain of the p75 receptor results in developmental cell death of numerous neuronal populations in transgenic animals (Majdan et al., 1997). A targeted deletion that removes this transcript by homologous recombination has been used as a strategy to generate a new line of p75-deficient mice (Dechant and Barde, personal communication). The analysis of this null mutation in the p75 gene will provide new insights into additional signaling functions of this receptor.

**LIGAND-DEPENDENT p75 CELL DEATH**

Several lines of evidence now firmly support that neurotrophins can actively kill cells through direct engagement of its p75 receptor. Both in vitro and in vivo evidence has indicated that neurotrophins and p75 are required for apoptosis of selective cell populations. Apoptosis of precursor cells was induced by NGF in developing retina and spinal cord and required binding to p75 expressed in the absence of the TrkA receptor (Frade and Barde, 1998; Frade et al., 1996). Administration of antibodies against NGF or p75 in chick embryos resulted in prevention of apoptosis of cells in the dorsal retina at early developmental ages (Frade et al., 1996), indicating that endogenous NGF causes the death of these retinal neurons.

In contrast to the model of NGF rescue through p75, other studies demonstrate that cultured trigeminal neurons at embryonic age E10 are killed by NGF through binding to p75 (Davey and Davies, 1998).
BDNF produced a survival effect, signaling through TrkB receptors in trigeminal neurons. In another paradigm, motor neurons subjected to axotomy or nerve crush were more susceptible to cell death with NGF treatment (Sendtner et al., 1998; Terrado et al., 1998). The sensitivity of motor neurons to NGF suggests that the cell death role of p75 is elicited upon injury or traumatic conditions. Similarly, cultured glial cells, such as fully differentiated oligodendrocytes, express elevated levels of p75 receptor and are effectively killed by NGF (Casaccia-Bonnefil et al., 1996).

The apoptotic effects of p75 are not only enhanced by NGF binding but, in certain restricted conditions, also by high concentrations of BDNF and other neurotrophins. Postnatal sympathetic neurons express p75 and TrkA, but are killed by BDNF-mediated activation of p75 (Bamji et al., 1998). The observations in cultured sympathetic neurons were further supported by an increased number of sympathetic neurons detected in BDNF-deficient mice and in a delay in sympathetic neuronal maturation. Motor neurons expressing p75 receptors are also sensitive to high concentrations of BDNF and NT-3 (Chu et al., 1998). While it may be argued that excessively high levels of neurotrophins do not occur in vivo, these results nevertheless indicate the potential of these proteins to elicit a cell death response (Table 2).

On the other hand, trigeminal neurons dependent on BDNF for survival are killed by NT-4 through binding to p75 (Agerman et al., 1999). Primary cell culture experiments demonstrated that p75 was necessary for neuronal cell survival promoted by BDNF, but NT-4 binding to p75 induced cell death. This remarkable set of observations indicates that p75 and Trk receptor can simultaneously influence life-and-death decisions in neurons depending upon which ligands are available.

An issue raised by these experiments is that although all neurotrophins (NGF, BDNF, NT-3, and NT-4) bind to p75 with similar affinity, each neurotrophin may exert different effects on cell function and viability through p75. For example, the effect of NGF on oligodendrocyte cultures could not be reproduced by similar concentrations of BDNF or NT-3. Furthermore, in PC12 cells treated with antisense oligonucleotides to downregulate TrkA expression, BDNF but not NGF can rescue cells from serum-withdrawal (Taglialatela et al., 1996). A very likely explanation for the effect of distinct neurotrophins in the oligodendrocyte system is the presence of TrkB and TrkC receptors in these cells (Cohen et al., 1996). An alternative explanation for cells not expressing other Trk receptors is the differential ability of neurotrophins to activate distinct signal transduction pathways (Carter et al., 1996; Carter and Lewin, 1997). This hypothesis is also supported by striking differences in the kinetics of binding and the degree of positive cooperativity of each neurotrophin to p75 (Rodriguez-Tebar et al., 1990, 1992). A related explanation may involve different adaptor molecules that are associated with the receptor.

**DOWNSTREAM CELL DEATH SIGNALING**

Much evidence presented here provides support for the hypothesis that p75 participates in the apoptotic process, but the components responsible for signaling remain to be identified. How does a ligand-independent mechanism lead to death? Apoptosis requires the activation of cysteine protease enzymes, or caspases (Alnemri et al., 1996; Ellis et al., 1991). This family of proteases, which now number 14 caspases (cysteine aspartyl proteases), shares similarities to ced-3, an essential cell death gene in Caenorhabditis elegans. The caspase function as initiator enzymes (caspase-1, -2, -8, -9) or effector caspases, such as caspase-3, to carry out the execution phase of apoptosis.

During p75-mediated apoptosis in oligodendrocytes, several caspase family members, including caspase-1 (ICE), caspase-2 (Nedd-2), and caspase-3 (CPP32/ Yama) are specifically activated (Gu et al., 1999). Caspase-1 was strongly activated by NGF and other injury stimuli, further supporting the association of specific caspases with inflammatory conditions. Interestingly, the p75 receptor, a member of the same TNF superfamily, does not activate caspase-8, suggesting that oligodendrocyte cell death induced by the p75 NGF receptor uses a quite different apoptotic mechanism from other TNF receptor members (see Fig. 2). It is known that Fas and TNF receptors activate caspase-8 by recruiting this enzyme to the receptor complex using the adaptor molecule FADD (Nagata, 1997).

The structural similarity of p75 to the death domain of the Fas and p55 TNF receptor suggested that the mechanism of apoptosis may be similar. However, this has not yet been functionally established. The death domain, a feature of nearly half of the members of the TNF receptor superfamily, functions primarily to provide a docking site for adaptor molecules, such as TNF Receptor Associated Factors (TRAFs), TRADD, and FADD (Fig. 2). Given the structural similarity between p75 and other TNF family members, it is conceivable that when p75 is expressed at high levels, apoptosis could result from inappropriate or normal interactions of p75 with FADD, TRADD, TRAFs, or related proteins in these families.

However, the mechanism of activation of p75 seems to differ from Fas and TNF receptors in that the p75 receptor is unable to self-associate and initiate a strong apoptotic signal in cells. Indeed, activation of p75 by NGF only resulted in apoptosis of specific cell types, either at distinct developmental stages or following stress (Casaccia-Bonnefil et al., 1996; Davey and Davey, 1996; Fadok and Badolato, 1998; Fadok et al., 1998). This action may be partially explained either by distinct ligand binding requirements (trimeric transmembrane ligands of the TNF family vs. soluble dimers of neurotrophin family) or by structural differences in the death domain. Although there are structural similarities between p75 and Fas death domain, there are also several notable differences, namely, the distribution of surface electrostatic charges and the position of the first helix. This structural information would predict that, although p75 may interact with common adaptor proteins, it may also bind novel signal transduction molecules. In addition, activation of caspase 8 (FLICE/Mach) by Fas has been shown to occur by recruitment of caspases, via FADD (Fig. 2) using a death effector domain (DED). A key feature of the DED domain is the presence and spacing of crucial hydrophobic residues.

The distribution of hydrophobic residues in p75 death domain structure and FADD death effector domain structure is strikingly similar, with higher homology between p75 and FADD than between Fas and FADD. The hydrophobic pattern in p75 death domain region...
suggested that it may directly recruit caspases to the membrane. However, caspase-8 was not activated upon ligand-dependent activation of p75 (Gu et al., 1999).

Many TNF receptor family members interact with TNF receptor-associated factors (TRAFs) to modulate JNK and NF-κB activity, as well as apoptosis. One molecule associated with p75 falls in the class of TNF receptor-associated factors TRAFs, which are required for NF-κB activation by TNF and IL-1 receptors (Rotte et al., 1995). So far six TRAF proteins have been identified that mediate signaling through receptors for TNF, CD30L, CD40L, and IL-1 (Arch et al., 1998). TRAF6 has been shown to interact with the p75 neurotrophin receptor (Khursigara et al., 1999). A specific interaction between p75 and TRAF6 was observed after transient transfection in 293T cells. The interaction was dependent upon binding to NGF and maximal at 100 ng/ml of NGF. Other neurotrophins, such as BDNF and NT-4, also promoted the association of TRAF6 with p75, but to a lesser extent. The binding of TRAF6 was localized to the juxtamembrane region of p75 and not the death domain (Fig. 3). The TRAF6 protein interacts with receptors and other adaptor proteins through a homology domain at the C-terminal region, the TRAF domain. TRAF6 also contains an N-terminal RING finger sequence, five zinc fingers, and a coiled-coil domain, which are required for TRAF6 signaling, including activation of JNK (Ishida et al., 1996). To assess the functional significance of this interaction, the NGF-mediated increase in the nuclear localization of the p65 subunit of NF-κB in cultured Schwann cells could be blocked by the introduction of a dominant negative form of TRAF6 in Schwann cells.

Another important molecule whose activity is induced by the p75 neurotrophin receptor is p53. The p53 tumor suppressor protein is involved in neuronal cell death, particularly after DNA damage or cellular stress. Activation of p75 in sympathetic neurons by BDNF leads to increased levels of p53 (Aloyz et al., 1998). The increase in p53 was also observed during withdrawal of NGF from sympathetic neurons, raising the interesting possibility that the pathway leading from the p75 receptor to elevated p53 levels may be similar to the apoptotic mechanism used by sympathetic neurons in the absence of NGF (Deshmukh and Johnson, 1995). Sympathetic neuronal cell death after NGF deprivation is characterized by an elevation in the level of the BAX protein and in the release of cytochrome c from mitochondria (Deshmukh and Johnson, 1998). Whether p75-mediated cell death in sympathetic neurons also induces these activities has not been determined.

A potential explanation to bring these two forms of cell death together is that serum or NGF withdrawal from cells may trigger changes in the p75 receptor structure or cell localization that adopt a cell killing role. Alternatively, the action of p75 in the absence of ligand binding may be explained by engagement of the cellular death machinery under conditions of overexpression.

It should be emphasized that the mere expression of p75 receptors is not sufficient to cause death. Consistent with this conclusion is the absence of cell death in cultured Schwann cells, which express very high levels of p75. Other death pathway proteins or specific cell competence factors may be required to engage the cell death program after p75 induction.

Detection of high levels of p75 in Schwann cells after nerve lesion or in culture (Lemke and Chao, 1988) suggested the expression of p75 may reflect a common reaction for glial cells, which is accelerated after nerve lesion or injury. The p75 receptor may be more accurately regarded as a stress receptor, similar in behavior to other TNF family members. Glial cells in vivo also possess the potential of expressing several other TNF receptor family members, including the Fas antigen (D’Souza et al., 1996; Dowling et al., 1996). Strikingly, p75-positive oligodendrocytes can be detected in white matter plaques from cases of multiple sclerosis (Dowling et al., 1997). Some of the p75+ oligodendrocytes found in these plaques are apoptotic, raising the possibility that some forms of NGF-mediated cell death may mimic the inflammatory or traumatic conditions that produce reactive glial cells.

**COMPETITIVE RECEPTOR SIGNALING**

The cell death/survival decisions depend upon whether Trk alone, Trk plus p75, or p75 alone are expressed. An interesting question regarding signal transduction mechanisms is whether activation of one neurotrophin receptor can be dominant over the other. This question has been addressed in studies where p75 expressing oligodendrocytes and compared to p75+TrkA+ oligodendrocytes (Yoon et al., 1998). Expression and activation of TrkA receptors in oligodendrocytes rescued these cells from cell death induced by the p75 receptor. TrkA-mediated rescue from apoptosis correlated with MAP kinase activation. At the same time, activation of TrkA in oligodendrocytes resulted in suppression of c-Jun kinase activity initiated by p75, while induction of NF-κB activity by p75 was unaffected. The results indicated there are at least two parallel and distinct p75 signaling pathways, NF-κB and JNK (Fig. 3). Induction of NF-κB through p75 was unaffected by TrkA action. However, activation of TrkA by NGF leads to down regulation of JNK activity. The ability of an inhibitor of the JNK pathway, CEP1347, to rescue NGF-mediated apoptosis provided support that JNK activity was critical for this activity (Yoon et al., 1998). Hence, TrkA-mediated rescue involves not only activation of survival signals but also simultaneous suppression of a death signal by p75.

A second messenger that is generated upon p75 activation is ceramide, which can activate JNK (Verheij et al., 1996; Westwick et al., 1995), but not the NF-κB activity. One possible explanation to account for Trk receptor actions upon p75 signaling is by suppression of sphingomyelin hydrolysis. A metabolite, sphingosine-1-phosphate, is stimulated by TrkA receptors, due to activation of the enzyme sphingosine-kinase (Edsall et al., 1997). In PC12 cells, TrkA kinase activity negates sphingomyelin hydrolysis induced by activation of the p75 receptor (Dobrowsky et al., 1994). While sphingosine-1-phosphate activates MAP kinase, it can also block JNK activity. One possibility is that production of sphingosine-1-phosphate through TrkA may be involved in the induction of MAP kinases, whereas JNK activity induced by p75 may rely upon ceramide. Therefore, it is plausible that activation of TrkA may lead to suppression of JNK activity, while allowing other pathways, such as NF-κB, to proceed.

The survival response to NGF appears to be mediated by competitive signaling between TrkA and p75 (Fig. 3).
This mechanism is not restricted to NGF, as BDNF signaling is also capable of interfering p75-mediated cell death through the TrkB receptor (Davey and Davies, 1998). This may take place at the level of receptor binding, in which p75 and Trk receptors participate in high-affinity site formation. This binding site may serve to recruit unique signaling substrates to the receptor complex. Another mechanism to account for receptor cross-talk is that phosphorylation events merge at points downstream of the ligand-receptor level to give an alternative outcome. This would imply that the two receptors may interact functionally. Co-expression of TrkA and p75 receptors resulted in selective down-regulation of potential stressed-induced signals by p75, such as the JNK pathway, and simultaneous upregulation of MAP kinases and the steps leading to NF-κB activation. The balance between the activities of different MAP kinase subfamilies appears to play a determining role in survival decisions. These results are similar to cell death induced in PC12 cells after NGF withdrawal, where JNK activity is also activated (Xia et al., 1995).

Another survival pathway involves the recruitment and activation of PI-3 kinase, which consists of a regulatory p85 subunit and a catalytic p110 subunit (Fig. 3). Activation of PI-3 kinase and its downstream serine/threonine kinase Akt is required for cell survival signaling by tyrosine kinase receptors, such as TrkA (Ulrich et al., 1998; Yao and Cooper, 1995). The direct phosphorylation of the initiator caspase, caspase-9, by Akt (Cardone et al., 1998) provides a link between tyrosine kinase signaling and the cell death machinery, and indicates that the downstream outcome is the sum of many signaling events emanating from different functional directions.

An important conclusion is that survival versus death decisions are dependent upon the integration of different signaling pathways. The apex of these cascades are transmembrane receptors that initiate intra-cellular communication. The strength and duration of receptor signaling and how each signal intersects with other pathways will determine how a survival signal is converted to an apoptotic outcome.

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