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PERSPECTIVE

Nerve Growth Factor-Independent Neuronal Survival: A Role for NO Donors

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ABSTRACT

Because of the limited therapeutic applications of nerve growth factor (NGF), there has been increasing focus on the development of pharmacological tools to bypass the requirement of NGF for the activation of the TrkA tyrosine kinase receptor neuronal survival pathway. In this issue of *Molecular Pharmacology*, the work by Culmsee et al. (p. 1006) shows that NGF-independent activation of TrkA by protein tyrosine phosphatase (PTP) inhibitors is only achieved when accompanied by release of nitric oxide (NO). This work identifies the integration of the NO/cGMP/protein kinase G (PKG) and NGF/TrkA pathways to induce activation of Akt and ERK1/2 and mediate neuronal survival in the absence of NGF. In addition, it underscores the potential therapeutic effects of ethyl-3,4-dephostatin (DPN), a stable analog of the naturally occurring PTP inhibitor dephostatin, which serves as a NO donor and protects neurons from apoptosis. This *Perspective* comparatively reviews two major signal transduction pathways that mediate NGF-independent neuronal survival by activating the TrkA pathway: the NO/cGMP/PKG and adenosine/G-protein-coupled receptor (GPCR) pathways.

Neurotrophins and their receptors are the major regulators of neuronal survival and apoptosis during development and after injury or nervous system disease (Chao, 2003). In vitro experiments have demonstrated the dependence of several neuronal populations on neurotrophins, particularly NGF. In addition to in vitro experiments, mice that are heterozygotes for NGF ($NGF^{+/-}$) show loss of neurons in the peripheral nervous system (Crowley et al., 1994) and decreased cholinergic innervation in the hippocampus (Chen et al., 1997), further substantiating the role for NGF in neuronal survival. The survival effects of NGF are mediated via the Trk receptor tyrosine kinase A (TrkA) (Fig. 1). NGF induces dimerization of TrkA, leading to the phosphorylation of the TrkA receptor. Trk phosphorylation induces the activation of three major intracellular signaling pathways: the PI3-kinase pathway (resulting in Akt phosphorylation), the Ras pathway (resulting in ERK1/2 phosphorylation), and the phospholipase Cγ pathway (Kaplan and Miller, 2000). The PI3-kinase/Akt pathway has been identified as the major pathway involved in NGF-dependent neuronal survival (Yao and Cooper, 1995). Akt suppresses apoptosis by directly targeting pro-apoptotic proteins, such as Bad, pro-caspase-9, and forkhead (Kaplan and Miller, 2000).

In addition to NGF, NO is also required for neuronal survival (Contestabile and Ciani, 2004). In vitro, dorsal root ganglia neurons undergo apoptosis upon nitric-oxide synthase inhibition (Thippeswamy et al., 2001). In vivo, genetic depletion of neuronal nitric-oxide synthase increases the susceptibility of dorsal root ganglia neurons to apoptosis (Keilhoff et al., 2002). NO can stimulate soluble guanylyl cyclase (sGC), the enzyme that catalyzes the conversion of GTP to cGMP. Cyclic GMP alters the activity of the cGMP receptor molecules (cGMP-dependent protein kinases, cGMP-regulated phosphodiesterases, cGMP-regulated ion channels). Similar to the NGF/TrkA signaling pathway, the NO/cGC survival signal is mediated by activation of Akt (Ciani et al., 2002).

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ABBREVIATIONS: NGF, nerve growth factor; TrkA, Trk receptor tyrosine kinase A; PI3, phosphoinositide 3; ERK, extracellular signal-regulated kinase; SGC, soluble guanylyl cyclase; PTP, protein tyrosine phosphatase; DPN, ethyl-3,4-dephostatin; GPCR, G-protein-coupled receptor; PKG, protein kinase G; Me-DPN, 4-*O*-methyl-ethyl-3,4-dephostatin; $p75^{NTR}$, p75 neurotrophin receptor.

Culmsee et al. (2005) reveals phosphorylation of TrkA as a novel point of cross-talk between the NGF and NO neuronal survival pathways. Culmsee et al. (2005) shed light on the molecular mechanism used by NO donors to rescue neurons from apoptosis. These investigators previously identified that inhibition of PTPs by orthovanadate stimulates the NGF/TrkA signaling pathway (Gerling et al., 2004). In their quest for more specific PTP inhibitors, they make the observation that only PTP inhibitors that serve as NO donors, such as DPN, induce phosphorylation of TrkA, activate Akt and ERK1/2, and are neuroprotective. By contrast, PTP inhibitors that do not release NO, such as methoxime-DPN, are not neuroprotective and do not activate TrkA. A striking observation in their work that links the NGF/TrkA with the NO/sGC survival pathways is the ability of sGC inhibition to block phosphorylation of TrkA and the neuroprotective effects mediated by NO donors.

The NGF/TrkA signaling pathway converges with pain-related ion channels to regulate NGF-mediated heat sensitivity of sensory neurons (Chuang et al., 2001) and with G-protein-coupled receptors (GPCR) to regulate neuronal survival (Lee and Chao, 2001). Activation of TrkA receptors in the absence of neurotrophins occurs upon activation with adenosine, which acts through A_{2A}, a GPCR. Adenosine or small ligands in the GPCR family that regulate tyrosine kinase activity in neural cells have been proposed as a strategy for promoting trophic effects during normal and neurodegenerative conditions (Lee and Chao, 2001; Rajagopal et al., 2004). In this system, through TrkA receptor phosphorylation, adenosine is capable of activating the PI3-kinase/Akt cascade, resulting in a survival response in PC-12 and hippocampal cells in the absence of NGF. The results of Culmsee et al. (2005) identify the NO/cGMP pathway as another signal transduction pathway that is integrated with NGF/TrkA signaling to modulate activation of PI3 Kinase/Akt pathway and subsequent neuronal survival. Both NO and adenosine can phosphorylate TrkA and exert neuroprotective effects in the absence of NGF. Both NO and adenosine-mediated neuronal survival independent of NGF depend upon activation of Akt. However, TrkA phosphorylation is indispensable for adenosine-mediated neuroprotection (Lee and Chao, 2001), but it is not necessary for NO-mediated neuroprotection (Culmsee et al., 2005). Therefore, adenosine-mediated neuroprotection occurs through Trk receptor signaling, whereas NO-mediated neuroprotection, as suggested by Culmsee et al. (2005), seems to occur by direct action of the NO/sCG/PKG pathway on the survival pathway of Akt and ERK1/2 downstream of TrkA. (Fig. 1). Because adenosine induces production of NO and prevents death in cardiomyocytes via a PKG pathway (Xu et al., 2005), cross-talk between adenosine and NO in neuronal cells could be envisioned.

Many researchers have sought to identify small molecules to mimic the effects of NGF. NGF, which is retrogradely transported to cholinergic neurons, prevents the death of such neurons in the basal forebrain after axotomy (Kromer, 1987). In the brains of patients with Alzheimer's disease, cholinergic neurons in the basal forebrain die, a process that contributes to the attention deficits and overall cognitive decline (Bartus et al., 1982). Given the vulnerability of cholinergic neurons in human disease, NGF is thus predicted to have therapeutic value in neurodegenerative diseases. However, properties of NGF, such as its hydrophilicity and its short half-life in the blood, make it inappropriate for crossing the blood-brain barrier through systemic delivery (Tuszynski, 2002), thus limiting its use as a neuroprotective drug. Alternative strategies have focused on either ex vivo gene

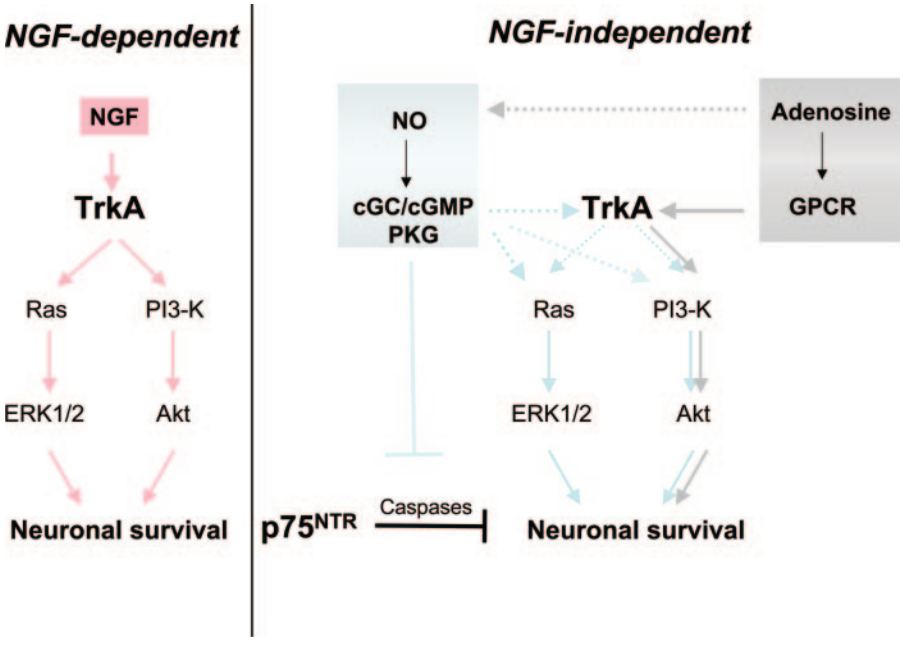

Fig. 1. NGF-dependent (left) and -independent (right) mechanisms for neuronal survival. A, NGF is the ligand of TrkA and induces its phosphorylation and the subsequent activation of the intracellular signaling pathways ERK1/2 and Akt, which are responsible for neuronal survival (Chao, 2003). B, Both NO and adenosine can mediate neuronal survival in the absence of NGF. Activation of TrkA phosphorylation occurs by both NO (Culmsee et al., 2005) and adenosine (Lee and Chao, 2001). TrkA phosphorylation is indispensable for adenosine but not for NO neuroprotection. In addition to activation of the pathways downstream of the TrkA receptor for NGF, NO might contribute to neuronal survival by inhibiting the apoptotic pathway mediated by the $p75^{NTR}$ NGF receptor (Lievremont et al., 1999). Because adenosine induces production of NO and prevents death in cardiomyocytes via a NO/cGMP/PKG pathway (Xu et al., 2005), cross-talk between adenosine and NO in neuronal cells could be envisioned.

delivery (Tuszynski et al., 2005) or on the design of biologically stable small molecules that can activate the NGF receptor signaling pathway so as to blunt neuronal loss in neurodegenerative diseases (Massa et al., 2003). Culmsee et al. (2005) identify two novel PTP inhibitors that mimic NGF: DPN and 4-*O*-methyl-ethyl-3,4-DPN (Me-DPN). In their previous work, they identified orthovanadate as a neuroprotective PTP inhibitor (Gerling et al., 2004). Orthovanadate has limited clinical applications because it is unstable in aqueous solutions and biological systems. In addition, orthovanadate is a wide-range PTP inhibitor, which limits specificity and safety. DPN and Me-DPN are stable analogs of the natural PTP inhibitor DPN and thus have potential clinical applications (Umezawa et al., 2003). Previous studies have shown that Me-DPN enhances NGF-induced differentiation in PC-12 cells (Fujiwara et al., 1997). In this system, Me-DPN induced differentiation only in the presence of NGF. In contrast, in the present study, DPN alone is sufficient to protect neurons from apoptosis, even in the absence of NGF. Therefore, stable DPN analogs bypass the requirement for NGF in neuroprotection through a NO/cGMP signal that activates TrkA, Akt and ERK1/2 in neuronal cells. Given their biological stability compared with either NGF or natural DPN, DPN analogs represent candidate therapeutic agents for activating the TrkA neuronal survival pathway.

Although DPN analogs are neuroprotective at low concentrations (1–10 μM) they are either inactive or even neurotoxic at higher concentrations (>100 μM). NO plays a major role in physiological processes in the nervous system, such as neuronal survival, plasticity, and synaptic activity. NO deprivation can kill a neuron, but elevation of NO is also neurotoxic. NO is released by activated glia in a variety of neurodegenerative diseases, including Alzheimer's disease, cerebral ischemia, Parkinson's disease, and multiple sclerosis. DPNs activate the NGF pathway through NO release and could possibly contribute to the endogenous levels of NO in brain neuropathology. Increases in NO concentration above physiological levels induce oxidative and nitrosative stress and lead to neuronal death. NO is therefore considered a "Janus" molecule that can exert both neuroprotective and neurodegenerative effects (Contestabile et al., 2003). However, in Alzheimer's type dementia, long-term administration of NO donors that slowly release NO combined with nonsteroidal anti-inflammatory drugs have shown protective effects, characterized by reduction of β-amyloid plaques (Burgaud et al., 2002). Therefore, identifying the neuroprotective concentration of DPN analogs as well as their contribution to the NO levels in the brain of different animal models of neurodegenerative diseases will determine their therapeutic potential.

Conclusions

Deprivation of NO is akin to deprivation of NGF in terms of ability to kill neurons. Culmsee et al. (2005) identify how the NO/cGMP and NGF/TrkA pathways converge to mediate neuronal survival. Their most important findings are: 1) activation of TrkA receptor and subsequent rescue from apoptosis in the absence of neurotrophins by specific PTPs that function as NO-donors, such as DPN; 2) activation of the survival signaling pathways of Akt and ERK1/2 by NO-donors in the absence of NGF; 3) induction of TrkA phosphorylation via a NO/sGC/cGMP and PKG pathways; and 4) biologically stable analogs for DPN with NO-donor activity could bypass the requirement for NGF and have pharmacological value as neuroprotectants.

The cross-talk of NO with NGF signaling pathways seems not to be limited to TrkA, but extends to the low affinity NGF receptor, p75 neurotrophin receptor ($p75^{NTR}$). Recent evidence has shown that NO regulates the expression of $p75^{NTR}$ (Peterson and Bogenmann, 2003). In turn, neurotrophins can induce iNOS in neurons via a $p75^{NTR}$/NF-κB pathway (Burke and Bothwell, 2003). NO can protect neurons from $p75^{NTR}$ mediated apoptosis by decreasing activity of caspases via a cGMP-dependent mechanism (Lievremont et al., 1999) (Fig. 1). Similar to NO, $p75^{NTR}$ is also a "Janus"-faced molecule: it can be neuroprotective by potentiating the TrkA signaling pathway or can induce apoptosis in the absence of TrkA (Casaccia-Bonnefil et al., 1999). Experiments to elucidate the convergence of NO signaling pathways with both TrkA and $p75^{NTR}$ receptors, will reveal mechanistic information on the balance between life and death as regulated by the interactions of gases and growth factors in neuronal cells.

References

Bartus RT, Dean RLI, Beer B, and Lippa AS (1982) The cholinergic hypothesis of geriatric memory dysfunction. *Science (Wash DC)* **217:**408–417.

Burgaud J-L, Ongini E, and Del Soldato P (2002) Nitric oxide-releasing drugs: a novel class of effective and safe therapeutic agents. *Ann NY Acad Sci* **962:**360–371.

Burke M and Bothwell M (2003) p75 Neurotrophin receptor mediates neurotrophin activation of NF-kappa B and induction of iNOS expression in P19 neurons. *J Neurobiol* **55:**191–203.

Casaccia-Bonnefil P, Gu C, Khursigara G, and Chao M (1999) p75 neurotrophin receptor as a modulator of survival and death decisions. *Microsc Res Technique* **45:**217–224.

Chao MV (2003) Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nat Rev Neurosci* **4:**299–309.

Chen KS, Nishimura MC, Armanini MP, Crowley C, Spencer SD, and Phillips HS (1997) Disruption of a single allele of the nerve growth factor gene results in atrophy of basal forebrain cholinergic neurons and memory deficits. *J Neurosci* **17:**7288–7296.

Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, and Julius D (2001) Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. *Nature (Lond)* **411:**957–962.

Ciani E, Virgili M, and Contestabile A (2002) Akt pathway mediates a cGMP-dependent survival role of nitric oxide in cerebellar granule neurones. *J Neurochem* **81:**218–228.

Contestabile A and Ciani E (2004) Role of nitric oxide in the regulation of neuronal proliferation, survival and differentiation. *Neurochem Int* **45:**903–914.

Contestabile A, Monti B, and Ciani E (2003) Brain nitric oxide and its dual role in neurodegeneration/neuroprotection: understanding molecular mechanisms to devise drug approaches. *Curr Med Chem* **10:**2147–2174.

Crowley C, Spencer SD, Nishimura MC, Chen KS, Pitts-Meek S, Armaninl MP, Ling LH, McMahon SB, Shelton DL, Levinson AD, et al. (1994) Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. *Cell* **76:**1001–1011.

Culmsee C, Gerling N, Landshamer S, Rickerts B, Duchstein H-J, Umezawa K, Klumpp S, and Krieglstein J (2005) Nitric oxide donors induce neurotrophin-like survival signaling and protect neurons against apoptosis. *Mol Pharmacol* **68:** 1006–1017.

Fujiwara S, Watanabe T, Nagatsu T, Gohda J, Imoto M, and Umezawa K (1997) Enhancement or induction of neurite formation by a protein tyrosine phosphatase inhibitor, 3,4-dephostatin, in growth factor-treated PC12h cells. *Biochem Biophys Res Commun* **238:**213–217.

Gerling N, Culmsee C, Klumpp S, and Krieglstein J (2004) The tyrosine phosphatase inhibitor orthovanadate mimics NGF-induced neuroprotective signaling in rat hippocampal neurons. *Neurochem Int* **44:**505–520.

Kaplan DR and Miller FD (2000) Neurotrophin signal transduction in the nervous system. *Curr Opin Neurobiol* **10:**381–391.

Keilhoff G, Fansa H, and Wolf G (2002) Neuronal nitric oxide synthase is the dominant nitric oxide supplier for the survival of dorsal root ganglia after peripheral nerve axotomy. *J Chem Neuroanat* **24:**181–187.

Kromer LF (1987) Nerve growth factor treatment after brain injury prevents neuronal death. *Science (Wash DC)* **235:**214–216.

Lee FS and Chao MV (2001) Activation of Trk neurotrophin receptors in the absence of neurotrophins. *Proc Natl Acad Sci USA* **98:**3555–3560.

Lievremont J-P, Sciorati C, Morandi E, Paolucci C, Bunone G, Della Valle G, Meldolesi J, and Clementi E (1999) The $p75^{NTR}$-induced apoptotic program develops through a ceramide-caspase pathway negatively regulated by nitric oxide. *J Biol Chem* **274:**15466–15472.

Massa SM, Xie Y, and Longo FM (2003) Alzheimer's therapeutics: neurotrophin domain small molecule mimetics. *J Mol Neurosci* **20:**323–326.

Peterson S and Bogenmann E (2003) Osmotic swelling induces p75 neurotrophin receptor (p75NTR) expression via nitric oxide. *J Biol Chem* **278:** 33943–33950.
Rajagopal R, Chen ZY, Lee FS, and Chao MV (2004) Transactivation of Trk neurotrophin receptors by G-protein-coupled receptor ligands occurs on intracellular membranes. *J Neurosci* **24:**6650–6658.
Thippeswamy T, McKay JS, and Morris R (2001) Bax and caspases are inhibited by endogenous nitric oxide in dorsal root ganglion neurons in vitro. *Eur J Neurosci* **14:**1229–1236.
Tuszynski MH (2002) Growth-factor gene therapy for neurodegenerative disorders. *Lancet Neurol* **1:**51–57.
Tuszynski MH, Thal L, Pay M, Salmon DP, U HS, Bakay R, Patel P, Blesch A, Vahlsing HL, Ho G, et al. (2005) A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nat Med* **11:**551–555.
Umezawa K, Kawakami M, and Watanabe T (2003) Molecular design and biological activities of protein-tyrosine phosphatase inhibitors. *Pharmacol Ther* **99:** 15–24.
Xu Z, Park S-S, Mueller RA, Bagnell RC, Patterson C, and Boysen PG (2005) Adenosine produces nitric oxide and prevents mitochondrial oxidant damage in rat cardiomyocytes. *Cardiovasc Res* **65:**803–812.
Yao R and Cooper GM (1995) Requirement for phosphatidylinositol-3 kinase in the prevention of apoptosis by nerve growth factor. *Science (Wash DC)* **267:** 2003–2006.

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