ANTIOXIDANTS & REDOX SIGNALING
Volume 5, Number 5, 2003

Forum Mini Review

Antiapoptotic and Pronecrotic Actions of Neurotrophins

BYOUNG J. GWAG[1–3] and SUN H. KIM[2,3]

ABSTRACT

Neurotrophins promote the differentiation, growth, and survival of neurons in the nervous system. Specifically, neurotrophins promote neuronal survival by interfering with programmed cell death or apoptosis. In addition to roles of neurotrophins as survival factors, neurotrophins can act as risk factors of neuronal injury under various pathological conditions. Neurotrophins markedly potentiate neuronal cell necrosis induced by activation of *N*-methyl-D-aspartate receptors, deprivation of oxygen and glucose, and free radicals. Moreover, prolonged exposure to neurotrophins results in widespread neuronal necrosis through free radical-mediated mechanisms. Whereas cellular and molecular mechanisms underlying antiapoptosis action of neurotrophins have been well documented, extensive study will be needed to delineate mechanisms for the neurotrophin-induced neuronal necrosis through activation of Trk tyrosine kinase receptors. *Antioxid. Redox Signal.* 5, 621–627.

INTRODUCTION

THE NEUROTROPHINS, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophic factor (NT)-3, and NT-4/5, are a family of proteins essential for development and maintenance of the nervous system. Neurotrophins are synthesized in and released from target cells, which are then bound to two types of cell surface receptors, the Trk tyrosine kinase receptors and the p75 neurotrophin receptor (p75NTR), internalized, and retrogradely transported to the neuronal cell body (for review, see 35). Neurotrophins act as differentiation, growth, and survival factors through binding to Trk receptors (for review, see 40). The Trk receptors contain docking sites, Y490 and Y785, for adaptor proteins that signal Ras, phosphoinositide 3-kinase (PI3-K), and phospholipase C-γ (PLCγ) to be activated to mediate the neurotrophic functions (40).

Although roles of p75NTR remain largely elusive, p75NTR as a member of the tumor necrosis factor receptor superfamily constitutes a low-affinity receptor for neurotrophins and can mediate apoptosis in sympathetic neurons, hippocampal neurons, and oligodendrocytes (4, 8, 55). Proapoptosis actions of p75NTR have been reported in embryonic retina, sympathetic neurons, trigeminal ganglion neurons, and basal forebrain cholinergic neurons *in vivo* (4, 20, 56). Ceramide and the c-Jun N-terminal kinase pathway have been reported as downstream signals underlying p75NTR-mediated apoptosis (8, 36).

Unfavorable neurotoxic actions of neurotrophins have been recently discovered. Neurotrophins can enhance and cause neuronal necrosis, the other form of neuronal death, besides apoptosis through activation of p75NTR. Trk receptors appear to mediate the pronecrotic action of neurotrophins. In this review, we will focus on mechanisms underlying the dark side of neurotrophins depending upon patterns of neuronal death, apoptosis and necrosis.

APOPTOSIS AND NECROSIS

Apoptosis and necrosis were originally defined according to histological and electron microscopic observation of morphological changes in the process of cell death (28). Necrosis or swelling necrosis is initiated by fulminant cytotoxic injury that causes the early collapse of plasma membrane while nuclear membrane is conserved. This is accompanied by mas-

Departments of [1]Neuroscience and [2]Pharmacology, [3]Center for the Interventional Therapy of Stroke and Alzheimer's Disease, Ajou University, Suwon, Kyungkido, Korea.

sive influx of Ca^{2+}, Na^{+}, Cl^{-}, and H_2O that results in swelling of the cell body and organelles. Necrosis is also characterized by scattering condensation of nuclear chromatin. In contrast, apoptosis is characterized by shrinkage of the cell body and organelles due to efflux of K^{+} and water. Margination, aggregation, and condensation of chromatin and collapse of nuclear membrane are observed in cells undergoing apoptosis. As plasma membrane integrity is maintained and dying cells are rapidly ingested by phagocytosis, inflammatory reactions are not observed during apoptosis.

Biochemical, pharmacological, and molecular criteria have been extensively investigated to define apoptosis. The chromatin cleavage at internucleosomal sites (DNA laddering) has been applied as evidence of apoptosis because it was observed in thymocytes and lymphoid cell lines undergoing apoptosis following exposure to glucocorticoid (59). However, DNA laddering is not sufficient to define apoptosis as it has been observed in the process of necrosis (14). Terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) stain and fluorescent DNA dyes (*e.g.*, Hoechst dye and propidium iodide) have been used to reveal cleavage and condensation of chromatin during apoptosis (16). As cells undergoing necrosis are accompanied by DNA fragmentation and scattering condensation of chromatin, they are also labeled by TUNEL stain and DNA dyes (48). Thus, caution should be given to define apoptosis and necrosis by analyzing patterns of DNA fragmentation and chromatin condensation. Recently, signaling pathways for induction, propagation, and execution of apoptosis have been extensively investigated. These are a group of cysteine proteases called caspases, members of the tumor necrosis factor receptor superfamily, members of the Bcl-2 family, and the adaptor proteins (for review, see 50).

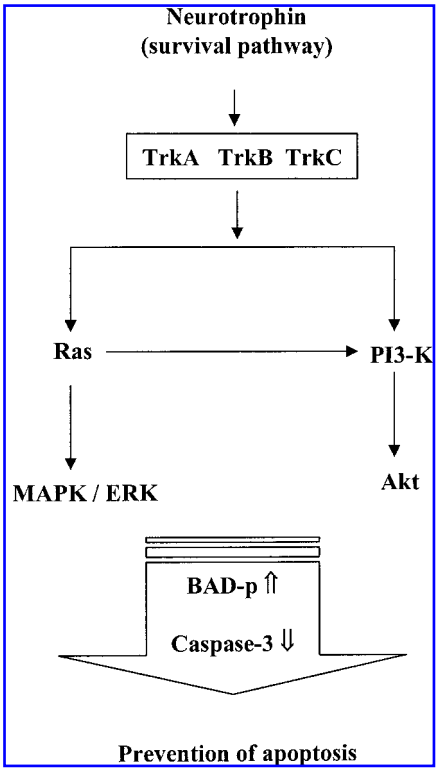

FIG. 1. Each neurotrophin binds to the appropriate Trk receptor, which in turn activates two survival pathways: Ras-mediated activation of MAPK/ERK survival pathway and PI3-K–mediated activation of Akt survival pathway. Both survival pathways appear to suppress apoptosis signals through BAD, caspase-9, and caspase-3.

ANTIAPOPTOTIC ACTIONS OF NEUROTROPHINS

Neurotrophins promote neuronal survival by preventing the developmentally programmed cell death or apoptosis in the nervous system (13). According to neurotrophic hypothesis, developing neurons extend their axons to target areas and can undergo further differentiation or degeneration dependent upon limiting amounts of neurotrophic factors that are released from the target cells. Thus, administration of neurotrophins promotes survival and differentiation of cultured neurons, including forebrain cholinergic neurons, mesencephalic dopaminergic neurons, and cortical neurons (1, 23, 31).

Trk-mediated signal transduction pathways have been well established underlying antiapoptosis actions of neurotrophins (Fig. 1). First, neurotrophins promote neuronal survival through the Ras signaling pathway. Phosphorylated Y490 binds to the Shc (Src homologous and collagen) adaptor protein, recruits Grb2 and the son of sevenless (SOS), and then activates Ras, which results in subsequent activation of the mitogen-activated protein kinases (MAPK)/extracellular signal-related kinase (ERK) pathways (17). The MAPK-activated kinases, p90 ribosomal S6 kinases (RSKs), induce phosphorylation of the proapoptotic protein BAD at serine 112 or the transcription factor cyclic AMP response element-binding protein (CREB) at serine 133, which prevents neuronal apoptosis (6). Second, PI3-K has been suggested as an additional route for antiapoptotic actions of neurotrophins. PI3-K is activated directly by interaction with Ras or indirectly by interaction with Grb2-associated binder-1 (Gab1) docking protein or insulin receptor substrate-1 and -2 (21, 60). Activated PI3-K produces inositol lipids that induce membrane recruitment of 3-phosphoinositide-dependent kinase-1 (PDK1) and its substrate, the serine/threonine protein kinase PKB (known as Akt) (57). Akt activated by PDK1 prevents apoptosis through phosphorylation of proapoptotic proteins, including BAD, caspase-9, and Forkhead, a transcription factor that induces apoptosis by increasing levels of Fas ligand (7).

PRONECROTIC ACTIONS OF NEUROTROPHINS

Potentiation of NMDA receptor-mediated excitotoxic necrosis

Glutamate mediates excitatory synaptic transmission through activation of ionotropic glutamate receptors sensitive to *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), or kainate. Although this excitatory transmission mediates normal information processing and neuronal plasticity, it plays a primary role in degeneration of neurons and oligodendrocytes in various neurological

diseases, including hypoxic-ischemia, trauma, and neurodegenerative diseases (24). Activation of ionotropic glutamate receptors allows entry of Ca^{2+} and Na^{+} through the plasma membrane. The massive entry and accumulation of the cations induce secondary influx of Cl^{-} and H_2O, resulting in marked swelling of the neuronal cell body within a few hours (9). Transmission electron microscopy reveals swelling of cytoplasmic organelles, including mitochondria, and scattering condensation of nuclear chromatin early in the process of excitotoxicity (19). Plasma membrane and cytoplasmic organelles are disrupted, but nuclear membrane remains intact. In contrast to apoptosis (shrinkage necrosis), membrane blebbing is not observed in the process of excitotoxicity. The morphological features suggest that neurons undergo necrosis following superfluous activation of NMDA, AMPA, or kainate receptors. Although DNA ladders, TUNEL-positive neurons, and chromatin condensation visible by DNA-binding fluorescence dyes have been used as evidence of apoptosis in excitotoxicity (3), they are all observed in the process of necrotic cell death (16, 48). Excitotoxic neuronal necrosis appears to propagate through signal transduction different from oxidative stress and apoptosis as neither antioxidants nor antiapoptosis agents (*e.g.,* inhibitors of macromolecule synthesis or growth factors) prevent excitotoxicity (19).

Controversial results have been reported on effects of neurotrophins against excitotoxicity. NGF and BDNF were shown to protect cultured hippocampal neurons from NMDA receptor-mediated excitotoxicity by reducing accumulation of intracellular free calcium (38). BDNF reduced death of cultured cerebellar granule cells from exposure to high concentrations of glutamate (37). Interestingly, pretreatment with BDNF potentiated NMDA receptor-mediated excitotoxicity in cultured hippocampal neurons and cortical neurons (31, 42). Although it remains to be elucidated how neurotrophins prevent or increase excitotoxic neuronal cell death, a novel hypothesis has been raised that neurotrophins prevent apoptosis, but potentiate NMDA receptor-mediated neuronal necrosis. In support of this, low or high doses of NMDA, as well as AMPA and kainate, induce neuronal cell death exclusively through necrosis in mature cortical cell cultures that is evident by electron microscopic analysis showing swelling of the cell body, irregularly scattering condensation of nuclear chromatin, and early collapse of plasma membrane (10). Neurotrophins potentiated the NMDA-induced neuronal necrosis. It is conceivable that administration of excitotoxins may induce neuronal apoptosis under apoptosis-preferring conditions (*e.g.*, immature neurons), which can be prevented by neurotrophins. Although further study will be needed to delineate mechanisms underlying the potentiation effects of neurotrophins on NMDA-induced neuronal cell necrosis, treatment with neurotrophins results in increased expression of NMDA receptor 2A subunit (NR2A) and neuronal nitric oxide synthase (NOS), which subsequently enhances Ca^{2+} influx and nitric oxide production, the key mediators of NMDA-induced neuronal death (Fig. 2) (22, 47).

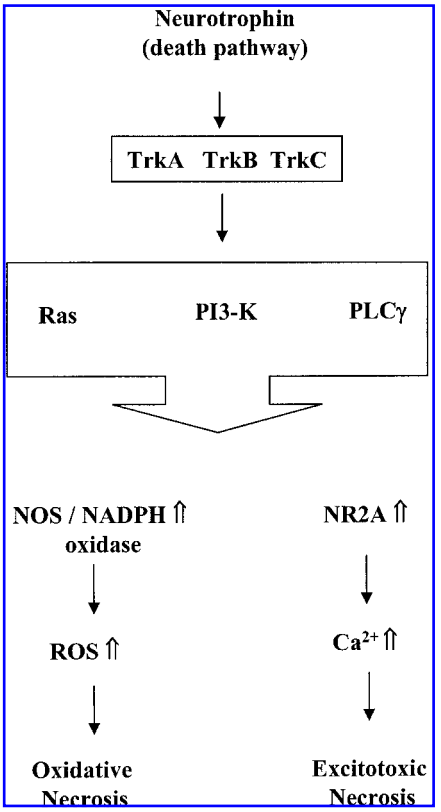

FIG. 2. Binding of neurotrophins to Trk receptors results in activation of three signaling molecules, Ras, PI3-K, and PLCγ, which is likely coupled to transcriptional and translational regulation of NOS, NADPH oxidase, and NR2A. Thus, intracellular ROS and Ca^{2+} will be accumulated over time following treatment with neurotrophins, potentiating or causing oxidative neuronal cell necrosis or NMDA receptor-mediated excitotoxic neuronal cell necrosis.

Potentiation of oxidative stress-induced neuronal necrosis

Oxidative stress generally describes a condition in which cellular antioxidant defenses are inadequate to completely detoxify the free radicals being generated, due to excessive production of reactive oxygen species (ROS), loss of antioxidant defenses, or typically both. A net increase of intracellular ROS can produce damage to lipids, protein, and DNA and eventually result in cell death. Several lines of evidence suggest that ROS act as triggers and mediators of apoptosis in various types of cells. First, ROS are produced in the process of apoptotic cell death (43). Second, ROS scavengers prevent apoptosis (33). Third, maneuvers increasing ROS can induce apoptosis (44). Finally, Bcl-2, the prototypical inhibitor of apoptosis as a mammalian protein homologous to *C. elegans* Ced-9, can prevent apoptosis and oxidative cell death by inhibiting formation of lipid peroxide and hydrogen peroxide (27). However, the central role of ROS as trigger and mediator of apoptosis has been challenged by opposing results. Oxidative stress can produce features of necrosis, as well as apoptosis. Bcl-2 does not protect oxidant-induced cell death in lymphoma cell lines and MN9D cells (11, 34). Moreover, apoptosis is induced in cells cultured in anaerobic conditions following exposure to staurosporine or interleukin-3 withdrawal, which is prevented by Bcl-2 (26). Thus, it is conceivable to postulate that ROS induce apoptosis or necrosis depending on cell types and circumstances.

The causative role of ROS for apoptosis has been reported in various populations of neurons (44), mostly turning to DNA ladders, DNA-binding fluorescence dyes, and the TUNEL method that do not differentiate apoptosis from necrosis. We also observed that mature cortical cell cultures exposed to prooxidants such as Fe^{2+} or buthionine sulfoximine (BSO) revealed DNA ladders, condensation of nuclear chromatin, and TUNEL-positive neurons (45). However, ultrastructural analysis of ROS-mediated neurotoxicity demonstrated occurrence of typical necrosis in cortical neurons evident by swelling of the cell body and mitochondria, scattering condensation of nuclear chromatin, and fenestration of plasma membrane prior to nuclear membrane (45). Levels of ROS were slightly increased in cortical neurons exposed to staurosporine, an agent inducing neuronal cell apoptosis in cortical cell cultures (41). The resulting neuronal apoptosis was not prevented by administration of antioxidants that completely blocked ROS production. Thus, ROS induce neuronal death exclusively through necrosis in mature cortical cell cultures.

Evidence has accumulated that neurotrophins protect neurons from oxidative stress. Administration of NGF reduces hydrogen peroxide-induced death in the PC12 rat pheochromocytoma cell line by increasing activity and expression of catalase and glutathione peroxidase (46). BDNF protects cultured dopaminergic neurons from the neurotoxins 6-hydroxydopamine and *N*-methyl-4-phenylpyridinium ion by increasing activity of glutathione reductase (49). Systemic administration of NGF, BDNF, and NT-4/5 reduces oxidative degeneration of striatal neurons in neonatal rats (30). Sympathetic neurons deprived of NGF undergo ROS-mediated apoptosis that depends on the activity of NADPH oxidase (53). Paradoxically, neurotrophins have been reported to enhance ROS-mediated neuronal death. Neurotrophins potentiate oxidative neuronal death in cortical and striatal cell cultures that is induced by administration of Fe^{2+}, BSO, or nitric oxide (25, 39). Although it remains to be elucidated how neurotrophins prevent and potentiate ROS-mediated neuronal cell death, we have hypothesized that the opposing effects of neurotrophins depend on patterns of neuronal cell death. In support of this, neurotrophins markedly potentiate oxidative neuronal death in cortical and striatal cell cultures that occurs exclusively through necrosis evident by morphological, biochemical, and pharmacological criteria (18, 39, 58). Further study will be needed to determine whether neurotrophins prevent the apoptosis component of ROS-induced neuronal death.

Neurotrophins: direct triggers of neuronal cell necrosis as prooxidants

Neurotrophins can induce neuronal cell death exclusively through necrosis. Extensive evidence indicates that neurotrophins enhance neuronal differentiation and survival. The neurotrophic activity of neurotrophins has been demonstrated in central and peripheral neurons that have been cultured in serum or defined media containing various antioxidants, such as putrescin and selenium (1, 12). When the effects of neurotrophins have been examined in mixed cultures of neurons and glia in serum-free media lacking antioxidants, controversial results have been observed. In particular, administration of neurotrophins for 1 day renders neurons highly resistant to apoptotic insults, but extremely sensitive to necrotic insults by excess activation of NMDA receptors or oxidative stress (31). One striking result stems from widespread neuronal cell death following prolonged (>2 days) exposure of mixed cortical cell cultures to BDNF, NT-3, and NT-4/5 (29). The neurotrophin-induced neuronal death is accompanied by swelling of cytoplasm and its organelles (*e.g.*, mitochondria), scattering condensation of nuclear chromatin, and early collapse of plasma membrane with relatively intact nuclear membrane, suggesting that neurotrophins induce direct neuronal death exclusively through necrosis. Intrastriatal injections of BDNF in adult rat also cause degeneration of striatal neurons around the injection site 2 days later.

ROS mediate neurotrophin-induced neuronal necrosis (NINN). Excitotoxicity and oxidative stress comprise two major routes for neuronal cell necrosis. Whereas neither NMDA receptor antagonists nor AMPA/kainate antagonists blocked NINN, concurrent administration of antioxidants completely blocked NINN. Moreover, the overall level of ROS was increased within 16 h selectively in cortical neurons following exposure of cortical cell cultures to BDNF, which was blocked with inclusion of antioxidants (29). This implies that neurotrophins induce direct neuronal death through ROS-mediated mechanisms. Interestingly, the protein synthesis inhibitor cycloheximide nearly completely blocked ROS production and neuronal necrosis by neurotrophins, suggesting that NINN requires ongoing macromolecular synthesis.

DNA microarray experiments were performed to screen putative target genes that would mediate prooxidant effects of neurotrophins. As expected, administration of BDNF increased mRNA expression of proteins associated with differentiation and proliferation within 8 h. BDNF also influenced mRNA expression of proteins that play a role in metabolism, signal transduction, and endocytosis. Among the BDNF-sensitive genes, we demonstrated that NADPH oxidase, a prooxidant enzyme generating superoxide from oxygen, mediated the prooxidant and pronecrotic effects of neurotrophins. First, administration of BDNF increased mRNA and protein levels of NADPH oxidase subunits selectively in cortical neurons prior to ROS production. Second, activation of NADPH oxidase was confirmed by translocation of the cytoplasmic subunits, p47-phox and p67-phox, into the plasma membrane. Consequently, superoxide was produced in neurons exposed to BDNF. Third, the selective inhibitors of NADPH oxidase, diphenylene iodonium or 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), prevented both ROS production and neuronal cell necrosis evolving after administration of neurotrophin.

The production of ROS has been observed in various cell types treated with growth factors, such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF), as well as NGF (2, 32, 52). Effector enzymes of growth factor receptors have been investigated underlying growth factor-induced production of ROS. Several lines of evidence suggest that PI3-K and the small GTP-binding protein Rac (Rac1or Rac2) are required and sufficient for the production of ROS in various cell types, including phagocytic cells after administration of PDGF or EGF (3, 51). PI3-K and Rac are also required for activation of NADPH oxidase (3, 54). Thus, it is

conceivable to postulate that PI3-K and Rac may act as signaling molecules underlying NADPH oxidase-mediated production of ROS by neurotrophins. ROS can then act as mediators of neuronal cell necrosis, as well as cell differentiation and proliferation.

CONCLUDING REMARKS

Neurotrophins enhance neuronal survival by interfering with programmed cell death or apoptosis in the process of normal development. The neuroprotective effects of neurotrophins have been demonstrated in animal models of neurological diseases, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and hypoxic-ischemia. As oxidative stress is a major cause of neuronal death in the neurological diseases above, the therapeutic application of neurotrophins should be compromised with their unwanted effects potentiating and inducing neuronal cell necrosis under certain circumstances. In particular, it is essential to determine the exact roles of excitotoxicity, oxidative stress, and apoptosis to neuronal death in acute and chronic neurodegenerative diseases and to delineate Trk-dependent signaling and molecular mechanisms underlying antiapoptosis and pronecrosis (*e.g.*, potentiation of NMDA receptor-mediated excitotoxicity and production of ROS) effects of neurotrophins.

ACKNOWLEDGMENTS

This work was supported by a National Research Laboratory grant from Korean Ministry of Science and Technology (BJG) and KOSEF through the BDRC at Ajou University (BJG).

ABBREVIATIONS

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF, brain-derived neurotrophic factor; BSO, buthionine sulfoximine; EGF, epidermal growth factor; ERK, extracellular signal-related kinase; MAPK, mitogen-activated protein kinase; NGF, nerve growth factor; NINN, neurotrophin-induced neuronal necrosis; NMDA, *N*-methyl-D-aspartate; NOS, nitric oxide synthase; NR2A, NMDA receptor 2A subtype; NT, neurotrophic factor; p75NTR, p75 neurotrophin receptor; PDGF, platelet-derived growth factor; PDK-1, 3-phosphoinositide-dependent kinase-1; PI3-K, phosphoinositide 3-kinase; PLCγ, phospholipase Cγ; ROS, reactive oxygen species; Trk, tyrosine kinase receptor; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling.

REFERENCES

1. Alderson RF, Alterman AL, Barde YA, and Lindsay RM. Brain-derived neurotrophic factor increases survival and differentiated functions of rat septal cholinergic neurons in culture. *Neuron* 5: 297–306, 1990.
2. Bae YS, Kang SW, Seo MS, Baines IC, Tekle E, Chock PB, and Rhee SG. Epidermal growth factor (EGF)-induced generation of hydrogen peroxide. Role in EGF receptor-mediated tyrosine phosphorylation. *J Biol Chem* 272: 217–221, 1997.
3. Bae YS, Sung JY, Kim OS, Kim YJ, Hur KC, Kazlauskas A, and Rhee SG. Platelet-derived growth factor-induced H_2O_2 production requires the activation of phosphatidylinositol 3-kinase. *J Biol Chem* 275: 10527–10531, 2000.
4. Bamji SX, Majdan M, Pozniak CD, Belliveau DJ, Aloyz R, Kohn J, Causing CG, and Miller FD. The p75 neurotrophin receptor mediates neuronal apoptosis and is essential for naturally occurring sympathetic neuron death. *J Cell Biol* 140: 911–923, 1998.
5. Bates B, Hirt L, Thomas SS, Akbarian S, Le D, Amin-Hanjani S, Whalen M, Jaenisch R, and Moskowitz MA. Neurotrophin-3 promotes cell death induced in cerebral ischemia, oxygen-glucose deprivation, and oxidative stress: possible involvement of oxygen free radicals. *Neurobiol Dis* 9: 24–37, 2002.
6. Bonni A, Brunet A, West AE, Datta SR, Takasu MA, and Greenberg ME. Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. *Science* 286: 1358–1362, 1999.
7. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, and Greenberg ME. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 96: 857–868, 1999.
8. Casaccia-Bonnefil P, Carter BD, Dobrowsky RT, and Chao MV. Death of oligodendrocytes mediated by the interaction of nerve growth factor with its receptor p75. *Nature* 383: 716–719, 1996.
9. Choi DW. Ionic dependence of glutamate neurotoxicity. *J Neurosci* 7: 369–379, 1987.
10. Choi DW. Excitotoxic cell death. *J Neurobiol* 23: 1261–1276, 1992.
11. Choi MS, Boise LH, Gottschalk AR, Quintans J, Thompson CB, and Klaus GG. The role of bcl-XL in CD40-mediated rescue from anti-mu-induced apoptosis in WEHI-231 B lymphoma cells. *Eur J Immunol* 25: 1352–1357, 1995.
12. Cohen A, Bray GM, and Aguayo AJ. Neurotrophin-4/5 (NT-4/5) increases adult rat retinal ganglion cell survival and neurite outgrowth in vitro. *J Neurobiol* 25: 953–959, 1994.
13. Davies AM. The role of neurotrophins in the developing nervous system. *J Neurobiol* 25: 1334–1348, 1994.
14. Enright H, Hebbel RP, and Nath KA. Internucleosomal cleavage of DNA as the sole criterion for apoptosis may be artifactual. *J Lab Clin Med* 124: 63–68, 1994.
15. Ferrari G, Anderson BL, Stephens RM, Kaplan DR, and Greene LA. Prevention of apoptotic neuronal death by GM1 ganglioside. Involvement of Trk neurotrophin receptors. *J Biol Chem* 270: 3074–3080, 1995.
16. Grasl-Kraupp B, Ruttkay-Nedecky B, Koudelka H, Bukowska K, Bursch W, and Schulte-Hermann R. In situ detection of fragmented DNA (TUNEL assay) fails to discriminate among apoptosis, necrosis, and autolytic cell death: a cautionary note. *Hepatology* 21: 1465–1468, 1995.
17. Greene LA and Kaplan DR. Early events in neurotrophin signalling via Trk and p75 receptors. *Curr Opin Neurobiol* 5: 579–587, 1995.

18. Gwag BJ, Lobner D, Koh JY, Wie MB, and Choi DW. Blockade of glutamate receptors unmasks neuronal apoptosis after oxygen-glucose deprivation in vitro. *Neuroscience* 68: 615–619, 1995.
19. Gwag BJ, Koh JY, DeMaro JA, Ying HS, Jacquin M, and Choi DW. Slowly triggered excitotoxicity occurs by necrosis in cortical cultures. *Neuroscience* 77: 393–401, 1997.
20. Hamanoue M, Middleton G, Wyatt S, Jaffray E, Hay RT, and Davies AM. p75-mediated NF-kappaB activation enhances the survival response of developing sensory neurons to nerve growth factor. *Mol Cell Neurosci* 14: 28–40, 1999.
21. Holgado-Madruga M, Moscatello DK, Emlet DR, Dieterich R, and Wong AJ. Grb2-associated binder-1 mediates phosphatidylinositol 3-kinase activation and the promotion of cell survival by nerve growth factor. *Proc Natl Acad Sci U S A* 94: 12419–12424, 1997.
22. Holtzman DM, Kilbridge J, Bredt DS, Black SM, Li Y, Clary DO, Reichardt LF, and Mobley WC. NOS induction by NGF in basal forebrain cholinergic neurones: evidence for regulation of brain NOS by a neurotrophin. *Neurobiol Dis* 1: 51–60, 1994.
23. Hyman C, Hofer M, Barde YA, Juhasz M, Yancopoulos GD, Squinto SP, and Lindsay RM. BDNF is a neurotrophic factor for dopaminergic neurons of the substantia nigra. *Nature* 350: 230–232, 1991.
24. Ikonomidou C and Turski L. Excitotoxicity and neurodegenerative diseases. *Curr Opin Neurol* 8: 487–497, 1995.
25. Ishikawa Y, Ikeuchi T, and Hatanaka H. Brain-derived neurotrophic factor accelerates nitric oxide donor-induced apoptosis of cultured cortical neurons. *J Neurochem* 75: 494–502, 2000.
26. Jacobson MD and Raff MC. Programmed cell death and Bcl-2 protection in very low oxygen. *Nature* 374: 814–816, 1995.
27. Kane DJ, Ord T, Anton R, and Bredesen DE. Expression of bcl-2 inhibits necrotic neural cell death. *J Neurosci Res* 40: 269–275, 1995.
28. Kerr JF. Shrinkage necrosis of adrenal cortical cells. *J Pathol* 107: 217–219, 1972.
29. Kim SH, Won SJ, Sohn S, Kwon HJ, Lee JY, Park JH, and Gwag BJ. Brain-derived neurotrophic factor can act as a pronecrotic factor through transcriptional and translational activation of NADPH oxidase. *J Cell Biol* 159: 821–831, 2002.
30. Kirschner PB, Jenkins BG, Schulz JB, Finkelstein SP, Matthews RT, Rosen BR, and Beal MF. NGF, BDNF and NT-5, but not NT-3 protect against MPP^+ toxicity and oxidative stress in neonatal animals. *Brain Res* 713: 178–185, 1996.
31. Koh JY, Gwag BJ, Lobner D, and Choi DW. Potentiated necrosis of cultured cortical neurons by neurotrophins. *Science* 268: 573–575, 1995.
32. Kreuzer J, Viedt C, Brandes RP, Seeger F, Rosenkranz AS, Sauer H, Babich A, Nurnberg B, Kather H, and Krieger-Brauer HI. Platelet-derived growth factor activates production of reactive oxygen species by NAD(P)H oxidase in smooth muscle cells through Gi1,2. *FASEB J* 17: 38–40, 2003.
33. Kruman I, Guo Q, and Mattson MP. Calcium and reactive oxygen species mediate staurosporine-induced mitochondrial dysfunction and apoptosis in PC12 cells. *J Neurosci Res* 51: 293–308, 1998.
34. Lee Y and Shacter E. Bcl-2 does not protect Burkitt's lymphoma cells from oxidant-induced cell death. *Blood* 89: 4480–4492, 1997.
35. Levi-Montalcini R. The nerve growth factor thirty-five years later. *In Vitro Cell Dev Biol* 23: 227–238, 1987.
36. Lievremont JP, Sciorati C, Morandi E, Paolucci C, Bunone G, Della VG, Meldolesi J, and Clementi E. The p75(NTR)-induced apoptotic program develops through a ceramide-caspase pathway negatively regulated by nitric oxide. *J Biol Chem* 274: 15466–15472, 1999.
37. Lindholm D, Dechant G, Heisenberg CP, and Thoenen H. Brain-derived neurotrophic factor is a survival factor for cultured rat cerebellar granule neurons and protects them against glutamate-induced neurotoxicity. *Eur J Neurosci* 5: 1455–1464, 1993.
38. Mattson MP, Lovell MA, Furukawa K, and Markesbery WR. Neurotrophic factors attenuate glutamate-induced accumulation of peroxides, elevation of intracellular Ca^{2+} concentration, and neurotoxicity and increase antioxidant enzyme activities in hippocampal neurons. *J Neurochem* 65: 1740–1751, 1995.
39. Park EC, Jou I, and Gwag BJ. Nerve growth factor potentiates the oxidative necrosis of striatal cholinergic neurons. *Neuroreport* 9: 687–690, 1998.
40. Patapoutian A and Reichardt LF. Trk receptors: mediators of neurotrophin action. *Curr Opin Neurobiol* 11: 272–280, 2001.
41. Pong K, Doctrow SR, Huffman K, Adinolfi CA, and Baudry M. Attenuation of staurosporine-induced apoptosis, oxidative stress, and mitochondrial dysfunction by synthetic superoxide dismutase and catalase mimetics, in cultured cortical neurons. *Exp Neurol* 171: 84–97, 2001.
42. Prehn JH. Marked diversity in the action of growth factors on *N*-methyl-D-aspartate-induced neuronal degeneration. *Eur J Pharmacol* 306: 81–88, 1996.
43. Prehn JH, Jordan J, Ghadge GD, Preis E, Galindo MF, Roos RP, Krieglstein J, and Miller RJ. Ca^{2+} and reactive oxygen species in staurosporine-induced neuronal apoptosis. *J Neurochem* 68: 1679–1685, 1997.
44. Ratan RR, Murphy TH, and Baraban JM. Oxidative stress induces apoptosis in embryonic cortical neurons. *J Neurochem* 62: 376–379, 1994.
45. Ryu BR, Ko HW, Jou I, Noh JS, and Gwag BJ. Phosphatidylinositol 3-kinase-mediated regulation of neuronal apoptosis and necrosis by insulin and IGF-I. *J Neurobiol* 39: 536–546, 1999.
46. Sampath D, Jackson GR, Werrbach-Perez K, and Perez-Polo JR. Effects of nerve growth factor on glutathione peroxidase and catalase in PC12 cells. *J Neurochem* 62: 2476–2479, 1994.
47. Small DL, Murray CL, Mealing GA, Poulter MO, Buchan AM, and Morley P. Brain derived neurotrophic factor induction of *N*-methyl-D-aspartate receptor subunit NR2A expression in cultured rat cortical neurons. *Neurosci Lett* 252: 211–214, 1998.
48. Sohn S, Kim EY, and Gwag BJ. Glutamate neurotoxicity in mouse cortical neurons: atypical necrosis with DNA lad-

ders and chromatin condensation. *Neurosci Lett* 240: 147–150, 1998.
49. Spina MB, Squinto SP, Miller J, Lindsay RM, and Hyman C. Brain-derived neurotrophic factor protects dopamine neurons against 6-hydroxydopamine and *N*-methyl-4-phenylpyridinium ion toxicity: involvement of the glutathione system. *J Neurochem* 59: 99–106, 1992.
50. Strasser A, Puthalakath H, Bouillet P, Huang DC, O'Connor L, O'Reilly LA, Cullen L, Cory S, and Adams JM. The role of bim, a proapoptotic BH3-only member of the Bcl-2 family in cell-death control. *Ann N Y Acad Sci* 917: 541–548, 2000.
51. Sundaresan M, Yu ZX, Ferrans VJ, Sulciner DJ, Gutkind JS, Irani K, Goldschmidt-Clermont PJ, and Finkel T. Regulation of reactive-oxygen-species generation in fibroblasts by Rac1. *Biochem J* 318: 379–382, 1996.
52. Suzukawa K, Miura K, Mitsushita J, Resau J, Hirose K, Crystal R, and Kamata T. Nerve growth factor-induced neuronal differentiation requires generation of Rac1-regulated reactive oxygen species. *J Biol Chem* 275: 13175–13178, 2000.
53. Tammariello SP, Quinn MT, and Estus S. NADPH oxidase contributes directly to oxidative stress and apoptosis in nerve growth factor-deprived sympathetic neurons. *J Neurosci* 20: RC53, 2000.
54. Thelen M, Dewald B, and Baggiolini M. Neutrophil signal transduction and activation of the respiratory burst. *Physiol Rev* 73: 797–821, 1993.
55. Troy CM, Friedman JE, and Friedman WJ. Mechanisms of p75-mediated death of hippocampal neurons. Role of caspases. *J Biol Chem* 277: 34295–34302, 2002.
56. Van der Zee CE and Hagg T. p75NGFR mediates death of cholinergic neurons during postnatal development of the neostriatum in mice. *J Chem Neuroanat* 14: 129–140, 1998.
57. Vanhaesebroeck B and Alessi DR. The PI3K-PDK1 connection: more than just a road to PKB. *Biochem J* 346 Pt 3: 561–576, 2000.
58. Won SJ, Park EC, Ryu BR, Ko HW, Sohn S, Kwon HJ, and Gwag BJ. NT-4/5 exacerbates free radical-induced neuronal necrosis in vitro and in vivo. *Neurobiol Dis* 7: 251–259, 2000.
59. Wyllie AH, Morris RG, Smith AL, and Dunlop D. Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis. *J Pathol* 142: 67–77, 1984.
60. Yamada M, Ohnishi H, Sano S, Nakatani A, Ikeuchi T, and Hatanaka H. Insulin receptor substrate (IRS)-1 and IRS-2 are tyrosine-phosphorylated and associated with phosphatidylinositol 3-kinase in response to brain-derived neurotrophic factor in cultured cerebral cortical neurons. *J Biol Chem* 272: 30334–30339, 1997.

Address reprint requests to:
Byoung J. Gwag
Department of Neuroscience and Pharmacology
Ajou University School of Medicine
Suwon, Kyungkido, 442-749, South Korea

E-mail: bjgwag@madang.ajou.ac.kr

Received for publication March 4, 2003; accepted June 30, 2003.

This article has been cited by:

1. Min Hee Kim, Sun Min Lee, Hyun Mo Koo. 2012. Ipsilateral and contralateral skilled reach training contributes to the motor function and brain recovery after left haemorrhagic stroke of rats. *Brain Injury* **26**:9, 1127-1135. [CrossRef]
2. James L. Franklin . 2003. Programmed Neuronal Death. *Antioxidants & Redox Signaling* **5**:5, 583-587. [Citation] [Full Text PDF] [Full Text PDF with Links]