

Part Two: Neurotrophic Factors - Their Role in Development, Trauma and Disease

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Neurotrophic factors have been shown to play a critical role in the normal development of neurons and appear to mediate a protective response to trauma and disease. In Part Two of this article, we briefly review the bioactivities of neurotrophic factors in these processes and provide a model for the role of neurotrophic factors in the response to ischemia and potentially other traumatic neural injuries. In Part One of this article, which appeared in Promega Notes 50, we provided an overview of these factors and their signaling pathways.

Functions of neurotrophic factors

Role of neurotrophic factors in development

The role of the neurotrophic factors in development has been investigated extensively in cultured cells from different brain regions. From such studies, it has become apparent that target-derived factors are important for normal synaptogenesis. Moreover, these studies indicate that survival of cultured embryonic neurons from different brain regions may require one or more neurotrophins. More recent advances have employed the use of growth factor-gene or receptor-gene knock-out mice. Although results with these animals must be interpreted with caution, these studies have demonstrated the importance of neurotrophic factors in normal development and, most interestingly, they highlight specific cell populations as developmental targets for different neurotrophins (1,2). For example, brain-derived neurotrophic factor (BDNF) has dramatic effects on the development of sensory neurons but very little apparent effect on motor neuron development, while neurotrophin-4 (NT-4) acts principally on motor neuron development. Nerve Growth Factor (NGF) is involved in the development of sympathetic and sensory neurons. Interestingly, the specificity of neurotrophic factors shown during development may not extend into the adult, where less specific effects often are displayed.

Role of neurotrophic factors in disease

The strong actions of neurotrophic factors on cell survival during *development* have led to a dramatic surge of interest in the possibility that these factors may ameliorate neurodegenerative diseases, as well as the possibility that defects in these factors may play roles in certain brain diseases. In spite of the excitement, there have been no definitive studies in two very important diseases - Alzheimer's disease and Parkinson's disease - in part because of the difficulty in creating suitable animal models for experimentation (3). However, encouraging results from studies on motor neuron disease in wobbler mice (4) and other diseases cautiously suggest that neural diseases such as amyotrophic lateral sclerosis and Parkinson's disease, among others, may be amenable to treatment with neurotrophic factors, including glial-cell-line-derived neurotrophic factor (GDNF; 5-7), as well as BDNF and ciliary neurotrophic factor (CNTF; 4). Of particular interest from the studies by Mitsumoto *et al.* (4) is the suggestion that neurotrophic factors from different gene families may act synergistically when administered in combination. However, it is important to re-emphasize that most current animal models only approximate human disease states, and that the results from animal models should be interpreted conservatively.

Protection by neurotrophic factors following traumatic injury

Many of the effects of trauma can be modeled experimentally by making controlled lesions in the brain and studying the effects of such damage on neurons that send to or receive from the damaged brain region. Neurons that normally would receive information from the damaged area experience a loss of input, while those that send information to the area suffer transection of their axons. Both classes of insult can lead to the rapid death of affected neurons. Such deafferentation or axotomy models have been used by many investigators to evaluate the neuroprotective effects of various neurotrophic factors.

The literature on this topic is too large for a concise review, but it pointedly reveals that generalizations regarding the effectiveness of any given neurotrophic factor in mitigating or preventing neural cell death after injury must be viewed cautiously. Many examples in the literature indicate that the neuroprotective effectiveness of a particular factor may be dramatic in certain neuronal systems of the brain but negligible in others, consistent with the cell specificity in development that was referred to above.

One of the first clear demonstrations of the efficacy of a neurotrophic factor in protecting neurons after injury was presented in 1990 by

Sendtner *et al.* (8). Their experiments involved cutting the facial nerves of newborn rats (axotomy) - a procedure that normally elicits rapid retrograde degeneration of facial nerve cell bodies. Three groups of rats were studied: in one group, only axotomy was performed; in the second group, the cut axons were treated with bovine serum albumin; and in the third group CNTF was applied to the severed axons. Axotomized facial neurons in the untreated group behaved as expected. Seven days after the axotomy, 77% of the facial neurons had died. Neurons in the serum-treated rats responded similarly - more than 80% had died within the first seven days. However, in marked contrast to both of these groups, 75% of the facial neurons of the CNTF-treated rats were still alive seven days after their axons had been cut. These results, and those of many other experiments, strongly suggest that administration of appropriate neurotrophic factors soon after injury can effectively protect neurons from death. However, very few studies have examined whether these protective effects last longer than one week, and therefore, it remains to be determined whether neuroprotective effects such as these are transient or permanent.

Neurotrophic factors and cerebral ischemia

In addition to trauma, another major assault on the brain is ischemia, which occurs when the brain is deprived of blood flow, such as in heart attacks and stroke. Several independent lines of investigation indicate an important role for the neurotrophins and other neurotrophic factors in this phenomenon.

Exogenous neurotrophic factors can ameliorate ischemia-induced cell death

Many studies have demonstrated that administration of BDNF, NT-4/5, NGF or FGF protects CNS neurons against focal or short-duration global ischemia *in vivo* (summarized in ref. 9). Focal ischemia is a good model for stroke and global ischemia for cardiac arrest. Most of these studies employed prolonged pre-exposure to the growth factors (1-3 days prior to the insult), as well as post-ischemic treatment. Recent results suggest that at least the prolonged post-ischemic administration of NT4/5 is necessary for protection (10). Stopping treatment after 1 day allowed the normal damage to develop 3 days later.

Endogenous neurotrophic factors may normally protect against ischemia

Two slightly different lines of study suggest that endogenous neurotrophic factors may be important, normal protective agents against ischemia. Firstly, mRNA for BDNF is strongly elevated after an ischemic insult. There is also a post-ischemic elevation of TrkB receptors, the specific receptors for BDNF and NT-4/5. The spatial pattern of their elevation is very interesting. TrkB up-regulation occurs only in cell populations that are resistant to ischemic damage. In the hippocampus, BDNF mRNA is elevated in the dentate granule cells, which are very resistant to ischemia, but is not elevated in the CA1 pyramidal cell region, which is very vulnerable to ischemia (9,11,12). Thus, factor and receptor up-regulation is associated with cell populations that do not succumb to the ischemic assault.

The second line of evidence for the importance of endogenous neurotrophic factors has emerged from studies demonstrating that brain cells can be very strongly protected against ischemic insults that would otherwise be lethal if the cells are pre-exposed to a short bout of ischemia or other insult. Most interestingly, these short pre-exposures elevate BDNF mRNA in regions of the brain that are typically vulnerable (13). Initially, pre-exposure had been thought to act via elevation of heat shock proteins. However, current evidence does not favor that hypothesis (14), but rather implicates the neurotrophic factors (13). While only correlative at this stage, these data, in conjunction with the demonstration of protection by exogenous neurotrophins, strongly support an intrinsic role for neurotrophins as protective agents against ischemia.

The mechanism by which ischemia or other insults increase neurotrophins is only partially understood, but it appears to involve the activation of glutamate receptors by glutamate released during ischemic insult (12). Neurotrophin synthesis has been shown to be activated by increased levels of intracellular calcium, resulting either from calcium entry through voltage-sensitive channels or glutamate-mediated NMDA channels (9).

Deleterious effects of neurotrophins - a caution

While exogenous growth factors show remarkable protective effects in many cases, this is not true in all instances. Indeed, neurotrophins may be deleterious in certain forms of ischemic insult. Results on cultured neurons have revealed that BDNF causes a very rapid increase in the calcium concentration in neuronal dendrites by opening calcium channels. This effect is completely blocked by the fungal metabolite K252a, indicating that the calcium influx results from tyrosine kinase activation (15,16). It would be expected that this calcium influx might be toxic to neurons during ischemia because of the postulated role of intracellular calcium as the damaging agent. Indeed, in cultured cells exposed to a normally sublethal ischemia, BDNF has been shown to transform the insult into a lethal one (17). This apparently anomalous, and important, finding is discussed below.

Possible protective mechanisms of neurotrophic factors: a speculative model for ischemia

During development, neurotrophins almost undoubtedly enhance cell survival by regulating the natural cell death process termed apoptosis (18). To better understand the actions of neurotrophins during ischemia, it will be crucial to determine what processes they

interrupt. Do neurotrophins arrest a classical cell necrotic process induced by fairly large elevations in cytosolic calcium, or do they arrest an apoptotic-like process possibly induced by smaller elevations in calcium or reduced ATP levels? *Evidence at this time suggests that the neurotrophins block an apoptotic-like process.*

Although still controversial, recent evidence indicates that apoptosis is a major component of the delayed neuronal death that occurs after short exposures to global ischemia and in the penumbra of focal ischemic insults (19,20) - the types of *in vivo* insults that respond to neurotrophins. Furthermore, a large series of studies on neuronal/glial cultures recently reported by Dennis Choi and co-workers (21) showed that there are several conditions in which inhibition of oxidative energy metabolism causes neuronal cell death by an apoptotic process. This was demonstrated by its apparent requirement for protein synthesis, its ability to induce DNA laddering, and by the ability of the proto-oncogene product Bcl-2 to block this process. BDNF is clearly able to protect against this form of damage, but is unable to protect against damage induced by a larger calcium influx, which has none of the characteristics of apoptosis (17,21). If this is correct, then the other form of ischemic cell death, which is frankly necrotic and is caused by more profound insults and thus higher calcium levels, may well be exacerbated by neurotrophins, possibly because of their ability to enhance calcium entry (17).

Molecules involved in protection by neurotrophins

What are the molecular products of neurotrophin action that protect ischemic neurons? This critical question cannot be answered at this point, but there are some tantalizing clues that bear on the general mode of action of neurotrophins in enhancing neuronal survival.

Apoptosis following neurotrophic factor withdrawal can be blocked by an inhibitor of the protease interleukin-1beta-converting enzyme (ICE); and ischemic cell death is strongly attenuated by calpain (calcium-activated neutral protease) inhibitors. The possibility that neurotrophins induce synthesis of a protease inhibitor to prevent cell damage is thus an attractive one (15).

Calcium is considered a very important mediator of cell damage and death. It is intriguing that NT-3, and to a lesser extent, BDNF cause a marked increase in mRNA for calbindin, a 28kD calcium binding protein, and also in the amount of calbindin in some cultured hippocampal neurons (22). Induction of such a binding protein might help protect against anoxic/ischemic damage. Possibly related to this finding is the demonstration that the elevation in cytosolic calcium, which normally occurs several hours after exposures of cultures to hypoglycemia, is eliminated by pre-exposure of the cultures to protective doses of NGF or FGF, even after the onset of glucose deprivation (23).

Finally, and most exciting, is the recent demonstration that treatment of cultured cortical cells with BDNF or NGF greatly increases the concentrations of two free-radical scavenging enzymes - superoxide dismutase and glutathione reductase - and markedly attenuates the increase in free radical concentrations that normally results from toxic glutamate exposure (9,24). Such an elevation in free radicals is also thought to be an important mediator of anoxic/ischemic cell damage (25).

The simple model presented in [Figure 1](#) might describe the role of neurotrophins during anoxia/ischemia and possibly other trauma.

Figure 1. A model for the role of neurotrophic factors during anoxia/ischemia and perhaps following traumatic injury to neurons. Panels A and B: Cellular responses to ischemia.

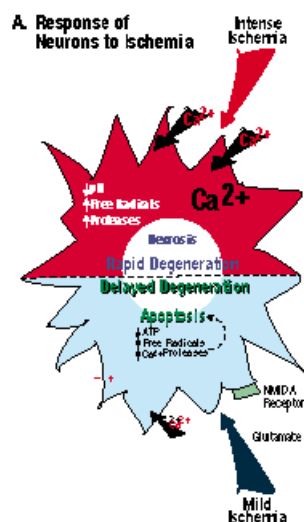


Figure 1. Panel A: For mild ischemic insults (blue arrow) that trigger delayed death, the condition below the dotted line prevails. The primary insult, decreased ATP and moderately increased cytosolic calcium levels cause accretion of free radicals and activation of calcium-dependent proteases, leading to apoptosis or other forms of delayed death. Either of these processes may be interrupted by the "survival proteins" listed in Panel C. For intense ischemic insults (red arrow) that produce rapid death (within 2-12 hours), the condition above the dotted line prevails. The rise in calcium is large, and there is a pronounced fall in intracellular

pH. These events lead to rapid degenerative processes in the cytoplasm and nucleus. These processes can be significantly accelerated by the increased calcium influx caused by the neurotrophic factors - the protective effects of neurotrophic factors would be of little value under these conditions. Thus, it seems possible that increased calcium entry (and possibly other factors) could convert a *mild* insult into an *intense* insult.

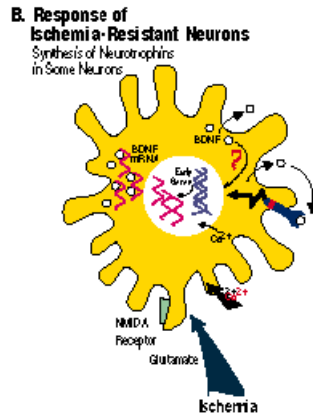


Figure 1. Panel B: Ischemia-resistant cells synthesize BDNF mRNA several hours after an ischemic insult (perhaps via activation of early genes), potentially triggered by calcium entering via glutamate-mediated NMDA or voltage-dependent channels. Once formed, the BDNF acts as described in Panel C. Actions may also be mediated by neurotrophic factor interactions with an intracellular receptor (dashed line).

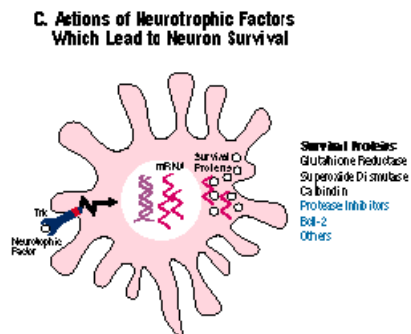


Figure 1. Panel C: Postulated actions of neurotrophic factors that lead to the *survival* of neurons. These actions may be effective against apoptotic as well as non-apoptotic (necrotic) cell death mediated by elevated calcium and/or free radicals. From left to right: neurotrophic factors interact with specific receptors, leading to tyrosine phosphorylation and the subsequent intracellular signaling cascade (reviewed in Part 1 of this article in *Promega Notes* 50; ref. 26). One rapid cytosolic action is to increase the calcium influx. Nuclear actions involve transcription of mRNAs for proteins which decrease damage by free radicals and calcium, and prevent apoptosis in, as of yet, unknown ways (Bcl-2). The "survival proteins" in black have been experimentally implicated in the biological response to neurotrophic factors; synthesis of the proteins in blue are speculative.

Conclusion

Although more than 40 years old, research on neurotrophic factors is, in fact, in an extremely rapidly growing phase. While many of the problems have now been defined, there are major questions to be answered in the areas of intra- and intercellular signaling, the mechanisms that underlie early cytosolic changes and gene activation, and the functional consequences of the broad range of neurotrophic factor activities. Moreover, caution must be exercised in generalizing from results using animal models of neural disease to human disease. However, given the profound effects of neurotrophic factors on neural development, function and survival, research in this area holds great promise for the future.

References

1. Klein, R. (1994) *FASEB J.* **8**, 738.
2. Jones, K.R. *et al.* (1994) *Cell* **76**, 989.
3. Eide, F.F., Lowenstein, D.H. and Reichardt, L.F. (1993) *Exp. Neurol.* **121**, 200.
4. Mitsumoto, H. *et al.* (1994) *Science* **265**, 1107.

5. Beck, K.D. *et al.* (1995) *Nature* **373**, 339.
6. Tomac, A. *et al.* (1995) *Nature* **373**, 335.
7. Yan, Q., Matheson, C. and Lopez, O.T. (1995) *Nature* **373**, 341.
8. Sendter, M., Kreutzberg, G.W. and Thoenen, H. (1990) *Nature* **345**, 440.
9. Lindvall, O. *et al.* (1994) *TINS* **17**, 490.
10. Chan, K. M. *et al.* (1994) *Abstracts Soc. Neurosci.* **20**, 179.
11. Lindvall, O. *et al.* (1992) *Proc. Natl. Acad. Sci. USA* **89**, 648.
12. Tsukahara, T. *et al.* (1994) *Abstracts Soc. Neurosci.* **20**, 616.
13. Kawahara, N. *et al.* (1994) *Soc. Neur. Abstracts* **20**, 1480.
14. Abe, H. and Nowak, T.S., Jr. (1994) *Soc. Neur. Abstracts* **20**, 1038.
15. Heumann, R. (1994) *Curr. Opinion. Neurobiol.* **4**, 668.
16. Berninger, B. *et al.* (1994) *Abstracts Soc. Neurosci.* **20**, 45.
17. Lobner, D. *et al.* (1994) *Soc. Neur. Abstracts* **20**, 442.
18. Franklin, J.L. and Johnson, E.M., Jr. (1994) *Phil. Trans. Roy. Soc. B. London* **345**, 251.
19. Martinou, J.-C. *et al.* (1994) *Neuron* **13**, 1017.
20. Kihara, S. *et al.* (1994) *Neurosci. Lett.* **175**, 133.
21. Choi, D.W. *et al.* (1994) *Soc. Neur. Abstracts* **20**, 604.
22. Ip, N.Y. *et al.* (1993) *J. Neurosci.* **13**, 3394.
23. Mattson, M.P. *et al.* (1993) *Exp. Neurol.* **124**, 89.
24. Lovell, M.A., Markesbery, W.R. and Mattson, M.P. (1994) *Soc. Neur. Abstracts* **20**, 611.
25. Siesjo, B. K. (1992) *J. Neurosurg.* **77**, 337.
26. Lipton, P. and Kalil, R. (1995) *Promega Notes* **50**, 18.

Ordering Information

Product	Size	Cat. #
NGF, 2.5S, Murine	100µg	G5141
NGF, 7S, Murine	100µg	G5151
rhNT-3	5µg	G1501
rhNT-4	5µg	G1511
rhBDNF	5µg	G1491
rrCNTF	5µg	G1481
rhFGF, Acidic	10µg	G5061
rhFGF, Basic	25µg	G5071
Anti-Human NGF mAb	50µg	G1131
Anti-Human NGF pAb	100µg	G1541
Anti-Human NT-3 pAb	200µg	G1651
Anti-Human NT-4 pAb	200µg	G1621
Anti-Human BDNF pAb	200µg	G1641
Anti-Rat CNTF pAb	200µg	G1631
Anti-Bovine FGF, Acidic IgG pAb	1mg	G5081
Anti-Bovine FGF, Fragment, Acidic IgG pAb	1mg	G5261
Anti-Bovine FGF, Basic mAb	500µg	G5271
Anti-FGF Receptor IgG pAb	250µg	G5102