Neurotrophic Factors: Two Are Better Than One

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Neurotrophic factors support the survival of neurons. The first and best characterized of these factors is nerve growth factor (NGF), without which sympathetic neurons die during development. NGF is but one member of a family of less well understood proteins—the neurotrophins—that includes brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4/5. Other potential neurotrophic molecules include ciliary neurotrophic factor (CNTF) and cytokines such as the fibroblast growth factors (FGFs), previously noted for their effects on nonneuronal cells. What are the physiological functions of these other molecules? It is attractive to suppose that each factor supports the survival of a single population of neurons, as is the case for NGF and sympathetic neurons. But in this issue of *Science*, Mitsumoto and co-workers show that the combined infusion of two neurotrophic factors—CNTF and BDNF—is better at arresting degeneration of motor neurons than either molecule alone (*1*).

The concept of a neurotrophic factor was originally based on the observation that the size of the target tissue regulated the number of innervating neurons that survived during development. The mediator of this target-neuron interaction was hypothesized to be a "neurotrophic" molecule synthesized and released by the target tissue. Through its action on sympathetic neurons, NGF is a prototypical, target-derived neurotrophic factor (*2*). Evidence that NGF is truly a neurotrophic factor is extensive: Exogenous NGF enhances survival of sympathetic neurons during developmental cell death; antibodies that block the action of NGF decrease neuronal survival during development; NGF is synthesized and secreted by targets of sympathetic neurons; and NGF is taken up by retrograde transport into sympathetic neurons.

Since the identification of NGF, the search has been intense for an equivalent neurotrophic factor for motor neurons. This effort has been fueled by the potential therapeutic importance of such a factor in halting the progressive degeneration observed in motor neuron diseases such as amyotrophic lateral sclerosis and spinal muscular atrophy. The classical biochemical fractionation techniques that had yielded NGF, BDNF, and CNTF through their ability to promote neuronal survival in vitro failed to produce a motor neuron factor. With hindsight, the reasons are clear. The in vitro screening assays for motor neurons are difficult to perform, because motor neurons must be somehow labeled or purified away from the other neurons and glial cells within the spinal cord. In addition, the target organ, muscle, is not sufficiently enriched for neurotrophic activity.

A more productive tactic has been to test the activity of known molecules on motor neurons. Many of these promote survival of motor neurons (*3*)—BDNF, CNTF, NT-3, NT-4/5, acidic and basic FGF, FGF-5, leukemia inhibitory factor, insulin-like growth factor (IGF)–1, and transforming growth factor–β. But not all studies have agreed. For example, Arakawa and colleagues found that CNTF and basic FGF, but not BDNF, supported chick motor neuron survival in cell culture (*4*), whereas, with a different protocol for purifying motor neurons, Henderson and colleagues found that BDNF (*5*), but not CNTF or basic FGF, supported chick motor neuron survival (*6*). Results from in vivo bioassays also sometimes differ from those of in vitro assays of trophic activity (*7*).

With this plethora of data, new questions arise. Which of these many candidate motor neuron trophic factors are physiologically relevant? Which therapeutically relevant? One clue was the partial effects that were often seen when molecules were tested on motor neurons, suggesting that no single factor was sufficient to rescue all motor neurons from cell death. In chick motor neuron cultures, 53 percent of the neurons survived with saturating concentrations of CNTF, and basic FGF supported survival of 51 percent, whereas the addition of CNTF plus basic FGF supported 100 percent survival. When trophic factors were applied to the chorioallantoic membrane, typically 20 percent of the motor neurons destined to die were rescued (*8*). In the *pmn* mouse, a model of motor neuron disease, the infusion of CNTF rescued 65 percent of the facial neurons (*9*). The report by Mitsumoto and co-workers shows that functional motor neuron loss in another model of motor neuron disease, the *wobbler* mouse, can be almost completely arrested by simultaneous treatment with CNTF and BDNF, suggesting that the two factors must synergize to promote complete neuronal rescue (or that there are two populations of motor neurons in the spinal cord, one that responds to CNTF and the other that responds to BDNF). The dramatic effects of BDNF and CNTF on motor neurons in *wobbler* mice are important and force a new level of complexity on the field. But they do not necessarily eliminate all other molecules as possible motor neuron trophic factors. Survival of neurons after the addition or overexpression of neurotrophic molecules shows only that the neurons have the capacity to respond to the molecules—but we still cannot be completely

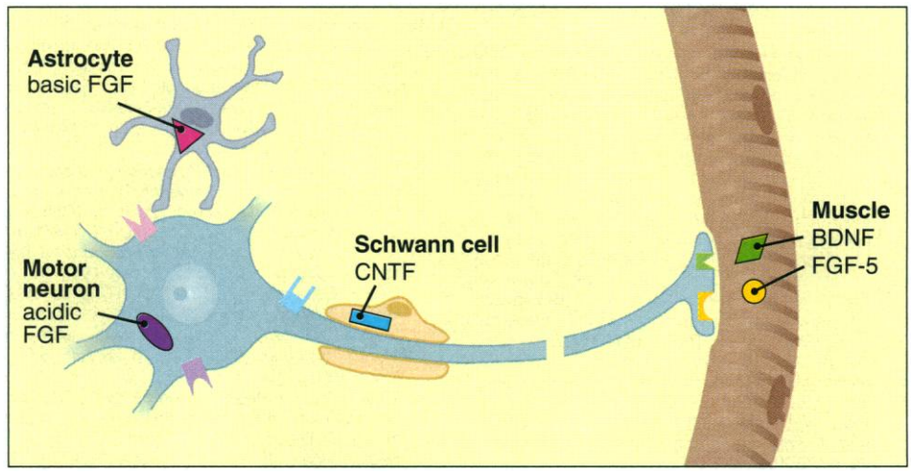

ILLUSTRATION: K. SUTLIFF

Compartmentalization of neurotrophic factors. Not all neurotrophic factors are located in the target organ, the muscle in the case illustrated here. CNTF is in the myelin surrounding the peripheral nerve, acidic FGF is in the motor neuron, basic FGF is in the astrocytes that surround the motor neuron cell body, and BDNF and FGF-5 are in the muscle. Other molecules may be in the interneurons, in sensory neurons, or in oligodendrocytes.

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certain of their physiological relevance.

With so many different molecules that support survival of a single population of neurons, where is the field of neurotrophic factors headed? Are we hopelessly entangled in a complex web, or is there a clear underlying principle to explain all? To decide, we must examine more carefully where candidate molecules are localized and how their levels are regulated during normal development, during axotomy-induced degeneration, and during spontaneous cell death in mutant mice. Candidate molecules and their receptors should be blocked or ablated. Indeed a number of gene-targeted "knockout" mice that lack CNTF, BDNF, or specific neurotrophin receptors (trk A, B, or C) are now available (*10–12*). In all of these cases, motor neurons appear to develop normally. However, the motor neurons in CNTF knockout mice atrophy 4 weeks after birth, resulting in a small but significant reduction in muscle strength. It is not yet known whether more dramatic reductions in motor neuron survival occur at later ages. Although the BDNF and trk B knockout mice appear to have normal motor systems, no one has yet examined whether the degeneration of the neurons in response to axotomy is accelerated or whether regeneration is slowed. Now that we know the potency of combining BDNF and CNTF in treating *wobbler* mice, the characterization of a double knockout mouse (that lacks both BDNF and CNTF) is all the more eagerly awaited.

The action of the neurotrophic factors may also depend on the type of cell death experienced by the motor neuron. Very little is known about the mechanisms of neuronal cell death or of the mechanism of cell rescue by trophic factors. Do motor neurons die via different mechanisms when death is target-dependent during development and when death is induced in adult neurons by axotomy? Do neurons in the *wobbler* mouse die by a mechanism similar to the way in which cells die in human neurodegenerative disease? Do neurotrophic factors that activate different receptors activate common signal transduction pathways, or do several parallel pathways end in cell rescue?

Perhaps optimal neural repair is achieved by the release of one or more molecules depending on the degree of the trauma experienced. Synergism of action between two molecules could then provide greater neuronal protection in the face of multiple lesions. In fact, distinct neurotrophic factors are present in different compartments around the motor neuron: CNTF is in the myelinating Schwann cells around the motor neuron axons, BDNF in the limb buds innervated by motor neurons, FGF-5 in differentiated muscle, acidic FGF in the motor neuron itself, and basic FGF in the astrocytes of the spinal cord (*3, 13, 14*). Because p75, the low-affinity neurotrophin receptor, is up-regulated in axotomized adult motor neurons, it is also possible that BDNF may be re-expressed in the muscle after peripheral nerve lesion. CNTF, acidic FGF, and basic FGF are sequestered intracellularly because they lack an amino-terminal signal peptide that would direct secretion of the molecule. They may be released only when the cells that sequester these molecules are lysed by injury. In contrast, FGF-5 may maintain the normal neuromuscular junction, while BDNF release after injury may ensure reinnervation of the muscle.

These are exciting times in the field of neurotrophic factors. The simple view of a single target-derived factor for each neuron has become more complex. Even sympathetic neuron survival in culture can now be achieved by at least four structurally distinct molecules: NGF, CNTF, acidic FGF (*15*), and basic FGF. Of these, NGF still reigns as the target-derived trophic factor that regulates developmental cell death, but the others may protect neurons from death induced by other means.

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