Nerve Growth Factor: Regulatory Role Examined

The biochemical signals guiding the complex processes and interactions of normal embryonic development are, for the most part, unidentified. An exception is nerve growth factor (NGF), a protein known for 25 years to participate in the growth, development, and maintenance of certain parts of the nervous system. Although the nature of that participation remains unclear, recent investigations have revealed that NGF resembles insulin both structurally and functionally and is therefore hormone-like in its activity. Nerve growth factor also appears to have a broader range of activities than once thought and may even be involved in such processes as carcinogenesis and wound healing.

The submaxillary (salivary) glands of male mice are an excellent source of NGF. The biologically active material isolated from these glands is a dimer of two identical polypeptides held together by noncovalent forces. Each polypeptide contains 118 amino acids and has a molecular weight of 13,259. Ralph Bradshaw, Ruth Angeletti, and William Frazier at Washington University School of Medicine in St. Louis, Missouri, determined the amino acid sequence of the NGF polypeptide and found that it resembled that of human proinsulin.

Proinsulin, the protein precursor of insulin, consists of a single polypeptide chain; it contains the A and B chains of the insulin molecule (insulin is composed of these two polypeptides held together by interchain disulfide bonds) separated by a third region called the C peptide. The C peptide is removed enzymatically during the conversion of proinsulin to insulin. According to Bradshaw, Frazier, and their co-workers, a number of physicochemical and chemical techniques used to assess the three-dimensional structure of proteins also indicated resemblances between NGF and insulin or proinsulin.

Even before determination of the amino acid sequence of NGF, several investigators had noted analogies between its biological effects and those of insulin. For example, NGF appeared to stimulate the synthetic and energy-producing processes of its target cells, just as insulin does. The fact that insulin acts by first binding to specific receptors on the surfaces of susceptible cells prompted Bradshaw and Frazier and also Eric Shooter of the Stanford University School of Medicine, Stanford, California, to investigate whether analogous receptors exist for NGF. They found that such receptors not only exist but that their distribution correlates with the biological activities of NGF.

Among these activities is enhancement of the growth of peripheral sensory and sympathetic neurons in embryos. (The sympathetic nervous system regulates such involuntary functions as the rate of heart beat and blood flow.) Elmer Bueker, now at the New York University School of Dentistry, New York, first observed that sensory nerve fibers invaded mouse sarcomas (malignant tumors) that had been transplanted into chick embryos. Rita Levi-Montalcini and Victor Hamburger of Washington University found that the tumors secreted a protein—NGF—that caused this growth stimulation of sensory neurons and also of sympathetic neurons both in vivo and in vitro.

The two types of neurons differ in their sensitivity to NGF. Sensory neurons of chick embryos exhibit their greatest response to it between the 7th and 12th days of embryonic life whereas sympathetic neurons respond during all developmental stages.

NGF and the Sympathetic Nervous System

The polypeptide is especially important for the development of the sympathetic nervous system. Levi-Montalcini and Pietro Angeletti, now at the Laboratory of Cellular Biology in Rome, Italy, and Stanley Cohen of Vanderbilt University in Nashville, Tennessee, found that antiserum against NGF, if administered to newborn mice and rats, selectively destroyed sympathetic nerve cells. The result was an animal that had been effectively and permanently "immunosympathectomized." Administration of the antiserum to adults caused less severe and reversible degenerative changes in sympathetic neurons, but NGF does seem to be necessary for maintenance as well as development of sympathetic neurons.

Although Levi-Montalcini found no effects of peripherally injected NGF on the brain, Anders Björklund and Ulf Stenevi of the University of Lund, Sweden, have shown that injections of NGF directly into the brain promote the regenerative growth of severed axons of certain central neurons. These neurons secrete norepinephrine, a chemical that transmits nerve impulses from neuron to neuron or from neuron to target organ. Sympathetic neurons also secrete norepinephrine. Thus, NGF, once thought to act only on peripheral neurons, also affects central nerve cells.

In vitro, NGF causes the formation of neurites, or projections from nerve cells by cultured sensory or sympathetic ganglia, frequently obtained from chick embryos (Fig. 1). Ganglia are groups of nerve cell bodies located outside the central nervous system; they contain nonneuronal cells in addition to neurons. If the cells of the ganglia are separated before culturing, the neurons soon degenerate and die unless NGF is added to the medium.

Neurite production by cultured ganglia is the classical assay of NGF. Bradshaw and Frazier found that NGF covalently attached to small insoluble beads would produce the same effect as free, soluble NGF. Since NGF made insoluble by attachment to beads had biological activity, transport of the material into the cells appeared unnecessary; these investigators concluded that NGF acts, as does insulin, by binding to receptors on the outer surface of cells.

Another way to study the binding of polypeptide hormones to receptors involves labeling the hormones with radioactive iodine and studying the properties of the binding of the labeled material by cells. Shooter found that cells prepared by mechanical disruption of embryonic sensory ganglia bound labeled NGF. The fact that only NGF and none of the other proteins tested, including proinsulin, could displace the radioactive NGF from the cells indicated that the binding was specific for NGF. According to Shooter, the binding sites could be saturated; that is, as the NGF concentration was increased, it eventually attained a level beyond which no additional binding was observed. The NGF concentration producing 50 percent saturation of the binding sites was the same as that producing maximal biological activity in

vitro. In a similar experiment, Bradshaw and Frazier observed a more complex type of binding that was not saturable and that was inhibited by insulin and proinsulin. The two investigators think that these discrepancies may be due to differences in their techniques and are collaborating in order to reconcile them.

In a study of the variation of NGF binding with time during chick embryo development, Bradshaw and Frazier found that the amount of NGF bound by sensory neurons increased between the 7th and 8th days and then declined sharply. In sympathetic ganglia, the total amount of binding remained approximately constant from the 8th to the 20th day of development. These patterns correspond to those of the neurons' sensitivities to NGF.

The Washington University investigators tested a number of other tissues of chick and rat embryos for NGF receptors. Brain and all peripheral organs innervated by the sympathetic nervous system have such receptors. They are not attributable to sympathetic nerve terminals in the tissues because they were detectable in the organs of animals whose sympathetic nervous system had been destroyed chemically. The organs that normally receive the greatest degree of sympathetic innervation had the greatest number of receptors.

The relationship between these receptors and the role of NGF in development is now under investigation. Bradshaw and his colleagues speculate that NGF bound to receptors on target organs could attract developing sympathetic neurons. Receptors on the neurons themselves may mediate the processes leading to and maintaining outgrowth of fibers from immature sympathetic nerve cells. Since NGF has now been found to affect the growth and regeneration of axons of central neurons, the receptors in brain could play a similar role.

The biochemical steps between binding of NGF to neuronal receptors and development of neurites have not been elucidated. Adenosine 3′,5′-monophosphate (cyclic AMP) is always a suspect for a "second messenger" in situations of this kind. Michael Young, Barry Arnason, and their colleagues at Harvard Medical School, Cambridge, Massachusetts, did find that a derivative of cyclic AMP that mimics the biological action of the nucleotide stimulates neurite outgrowth from sensory ganglia. Because of differences in the action of the derivative and of NGF itself, however, they concluded that the two materials produced their effects by different mechanisms and that cyclic AMP was not the "second messenger" for NGF.

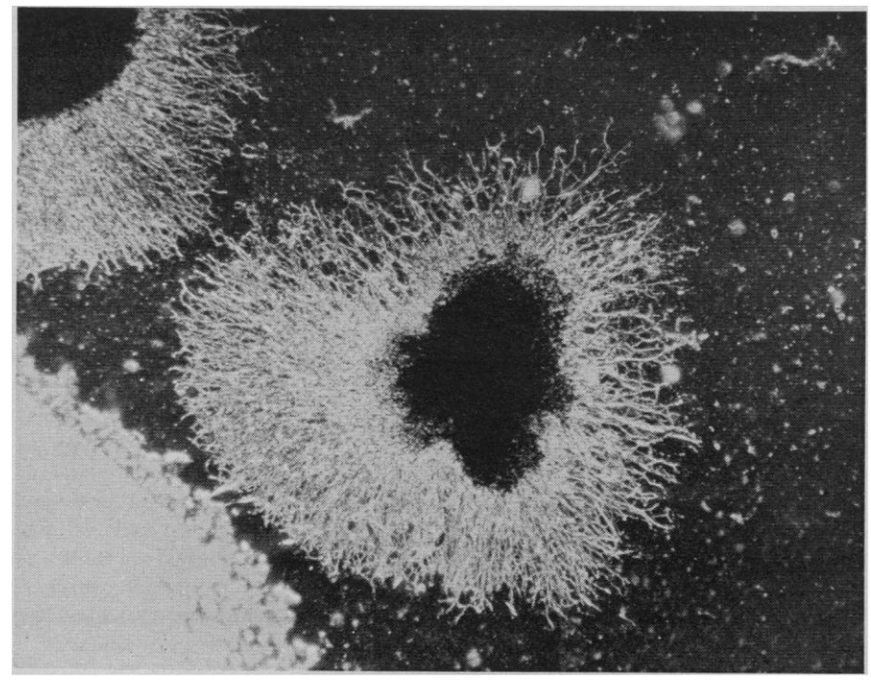

Fig. 1. Neurite outgrowth from cultured sensory ganglia of chick embryos. The outgrowth is directed predominantly toward the mouse fibroblasts (L cells) that lie within the left lower corner of the photomicrograph (× 600). [Source: Michael Young and Barry Arnason, Harvard Medical School]

Bradshaw, Frazier, and their co-workers observed no increase in intracellular cyclic AMP as a result of incubation of embryonic sensory ganglia with NGF, nor did NGF stimulate adenylate cyclase, the enzyme that catalyzes the synthesis of cyclic AMP from ATP. These results are again analogous with those for insulin, which does not act through the mediation of cyclic AMP. Insulin does increase production of guanosine 3′,5′-monophosphate, and this possibility is now under investigation for NGF.

The physiological source of NGF has puzzled investigators since Ian Hendry, now at Australian National University in Canberra, and L. L. Iverson of Cambridge University in England showed that the submaxillary gland could not be the sole source in mice. In the male mouse, this gland contains large quantities of NGF—approximately ten times that in the submaxillary glands of females and up to 1000 times more than in other tissues, including the heart, spleen, and kidney. It is unclear whether NGF is synthesized by male mouse submaxillary glands or merely stored there. Hendry and Iverson found that, after removal of these glands, the concentration of NGF in plasma decreased at first but then gradually increased until it returned to normal after about 2 months. Thus, other tissues must also synthesize it.

Among the candidates for this role are glial cells and fibroblasts. Glial cells are found in close association with neural cell bodies in ganglia and the brain. Their exact function is unknown, but they are generally thought to support and maintain neuronal function.

Silvio Varon and his colleagues at the University of California at San Diego observed that nonneuronal cells of ganglia—probably glial cells—were both necessary and sufficient for the continued viability of cultured ganglionic neurons. If enough of these nonneuronal cells were added to the cultures, added NGF was not needed to maintain the neurons. On the other hand, NGF alone was inadequate for neural maintenance. Other cell types, including mouse fibroblasts (3T3 cells) and kidney cells, could support neuronal viability in the presence of added NGF but only ganglionic nonneuronal cells could do so without added NGF.

In these experiments, it appeared that the glial cells acted by releasing an unknown agent or agents. Since antiserum against mouse submaxillary gland NGF

drastically impaired the survival of neurons cultured in the presence of optimal numbers of glial cells, Varon thinks that NGF is involved. The antiserum was active either when it was added directly to the cultures or when the glial cells were treated with it before being added to the cultures. This raises the possibility that the antibody interacts directly with the nonneuronal cells by combining with NGF-like antigens on their surfaces. According to Varon, glial cells could either be the source of NGF required by neurons or the NGF could somehow facilitate the activities by which glia support and maintain neurons.

Fibroblasts also secrete NGF, according to Young and Arnason. Their original observation was serendipitous. They attempted to use mouse L cells—a line of malignant fibroblasts—as a control in an experiment with NGF and unexpectedly found that the L cells themselves stimulated neurite outgrowth (Fig. 1). They determined that these cells secreted a material immunologically similar to, if not identical with, the NGF of male mouse submaxillary glands. Another line of fibroblasts, 3T3 cells, stimulated formation of neurites; 3T3 cells transformed by a oncogenic virus had the same effect.

Since all of these cell lines are in some way abnormal, Young and Arnason tested a primary culture of normal chick embryo fibroblasts. These too secreted a biologically active NGF that reacts with antibody to pure mouse NGF. Consequently, the investigators have proposed that secretion of NGF may be a general property of fibroblasts and that these cells may be a physiological source of the polypeptide.

Young and Arnason are now investigating whether NGF has more general effects in addition to those on nervous tissue. Mouse L cells secrete chemical factors that confer on the culture medium the capacity to stimulate the growth of other, unrelated cells such as baby hamster kidney cells or macrophages. (Macrophages are cells that are involved in inflammation, infection, and wound healing, and that may have a role in immune defenses against cancer.) Young and Arnason are attempting to determine whether NGF is one of the chemicals producing these growth effects.

Fibroblasts are prominent cell components of granulation or wound-healing tissue. Levi-Montalcini and Pietro Angeletti observed that granulation tissue produces a substance—probably NGF—that promotes nerve growth in vitro. If fibroblasts in wound-healing tissue release NGF as they do in culture, then NGF might be involved in wound healing. Proof that NGF activates macrophages, which are necessary for wound healing, would support this hypothesis.

Wound healing and tumor growth have a common requirement for increased blood supplies to meet their heavy energy requirements. According to Judah Folkman of Harvard Medical School, tumors can enhance their own vascular supply by secreting tumor angiogenesis factor (TAF) to stimulate the formation of blood vessels. Young and Arnason think that there could be a relationship between NGF and TAF. They found that cultured human glioblastoma cells (a glioblastoma is a tumor containing immature glial cells) produced a substance that reacts with antibody to mouse NGF. This tumor is known to secrete TAF. Young and Arnason are now investigating whether NGF has the capacity to stimulate the growth of blood vessels. Their early results look promising. If these results are confirmed, they will show that NGF participates in a number of processes involving growth and development, both in the nervous system and out of it.

—JEAN L. MARX

!Kung Hunter-Gatherers: Feminism, Diet, and Birth Control

If results from recent studies of the !Kung* people apply to other societies, anthropologists may now have some new clues as to the social, dietary, and demographic changes that took place during the Neolithic Revolution when people forsook lives of hunting and gathering and began to farm and to keep herds of domestic animals. The !Kung have lived as hunters and gatherers in the Kalahari Desert of South Africa (Fig. 1) for at least 11,000 years; but recently they have begun to live in agrarian villages near those of Bantus. Investigators who are documenting this change find that, among other things, the settled !Kung women are losing their egalitarian status, the children are no longer brought up to be nonaggressive, and the size of the !Kung population is rapidly increasing rather than remaining stable.

The !Kung's very existence is anomalous since they have lived by hunting and gathering since the Pleistocene. In his archeological studies, John Yellen of the Smithsonian Institution in Washington, D.C., finds artifacts from Late Stone Age hunter-gatherers, of about 11,000 years ago, at the same water holes where modern !Kung set up camp (Fig. 2). According to Yellen, these prehistoric hunter-gatherers even hunted the same animals as the contemporary !Kung, including the nocturnal springhare which must be hunted by a special technique because it spends its days in a long deep burrow.

As recently as 10 years ago, many of the !Kung still lived by hunting and gathering. Now, however, less than 5 percent of the 30,000 !Kung live in this way; the remainder live in agricultural villages. This period of rapid social change coincided with extensive study of these people by numerous investigators throughout the world and from many disciplines.

It is difficult to distinguish between changes due to settling down and changes due to acculturation to Bantu society. Investigators have drawn on extensive long-term studies of the nomadic !Kung in their documentation of the effects of the !Kung's adoption of an agrarian life, but cannot conclusively state the causes of these effects.

One aspect of the settled !Kung society that has aroused considerable interest among social scientists is the role of women. Patricia Draper of the University of New Mexico reports that !Kung women who belong to the nomadic bands enjoy higher status, more autonomy, and greater ability to directly influence group decisions than do sedentary !Kung women. This loss of equality for the agrarian women, Draper believes, may be explained in terms of the social structure of nomadic, as compared to sedentary, groups.

Draper postulates that one reason for the higher status of !Kung hunter-gatherer women is that the women contribute, by gathering, at least 50 percent of the food consumed by a band. Since food gathered by women is so impor-

* The exclamation point refers to an alveolar-palatal click. The tongue tip is pressed against the roof of the mouth and drawn sharply away, producing a hollow popping sound.