NGF and Alzheimer's: Hopes and Fears

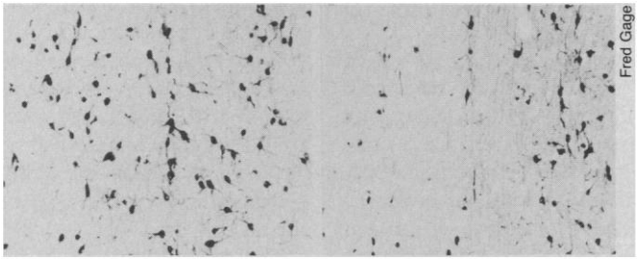
Nerve growth factor is headed toward clinical trials as a possible therapy for Alzheimer's disease—if concerns that it could do more harm than good can be resolved

RESEARCHERS MAY BE ON THE VERGE of beginning a new era in the treatment of degenerative brain diseases such as Alzheimer's. They are moving, albeit slowly and cautiously, toward initiating clinical trials to determine whether nerve growth factor (NGF) can reverse, or at least slow, the mental deterioration of Alzheimer's patients. "We're all very excited" says Franz Hefti, whose research helped to show that brain neurons could respond to the growth-stimulating effects of NGF.

If all goes well—and even the most ardent supporters of such trials concede that this is still a very big if—tests in human patients could begin in a year or two, according to Zaven Khachaturian, associate director of the National Institute on Aging.

Neurobiologists are turning to NGF because none of the other experimental therapies tried to date have been able to stop the mental deterioration of Alzheimer's patients and their inexorable progression to death. And animal studies have suggested that NGF might be able to prevent the nerve cell destruction that neurobiologists think causes the patients' memory losses. But despite the excitement about the idea of using NGF for Alzheimer's therapy, everyone is being very cautious—and with good reason.

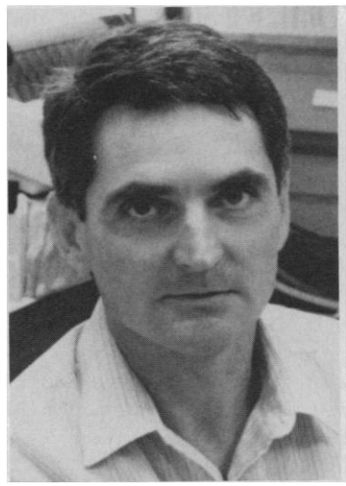
NGF is a "neurotrophic factor," a naturally occurring protein that is necessary for the growth and maintenance of certain types of nerve cells. Although it is the first such factor to be nearing clinical testing, others have been identified, and neurobiologists expect to find still more. Any of them might be potentially valuable for treating currently incurable conditions, such as Alzheimer's or Huntington's disease, that are caused by brain neuron degeneration. A disaster with NGF might jeopardize the entire field of research, Khachaturian says.



Fred Gage

Engrafting brain. *Putting NGF-producing cells into brain (left) prevents neurons (stained black) from degenerating after being cut.*

And there is scientific cause for concern. Some researchers have evidence that abnormal nerve cell growth may actually contribute to the pathological changes in Alzheimer's brains (also see box on p. 409). "If you feed the frenzy, so to speak, you may make things worse," says Carl Cotman of the University of California at Irvine. He notes, however, that the desperate straits of Alzheimer's patients, which may now include as many as 4 million people in the United States, might nonetheless justify the risk.



Franz Hefti: *"We are dealing with an entire new concept."*

Until about 5 years ago, no one was even looking at NGF as a potential Alzheimer's treatment. The reason was simple: It was not supposed to act in the brain at all. Rita Levi-Montalcini, who was then working with Viktor Hamburger at Washington University in St. Louis, discovered NGF around 1950 and found that it was needed for the development and maintenance of a type of peripheral nerve cell that transmits signals by releasing a chemical called norepinephrine. Everyone assumed, naturally enough, that it would act on comparable neurons in the brain; when they could find no such effects, they concluded that its action was limited to peripheral neurons.

In the mid-1980s, however, much to everyone's surprise, researchers, including Hefti, who then worked in Hans Thoenen's lab at the Max Planck Institute for Psychiatry in Munich, and Fred Gage of the University of California, San Diego, found that NGF has trophic effects on brain neurons that release the neurotransmitter acetylcholine (*Science*, 13 June 1986, p. 1341).

They showed, for example, that if they sever the cholinergic (acetylcholine-secreting) nerve tract connecting two brain areas, the basal forebrain and the hippocampus, in rats, the neurons degenerate in about 2 weeks. But injecting NGF into the animals' brains prevents that degeneration. "There's no doubt," says Hefti, who is now at the University of Southern California in Los Angeles, "that these cholinergic neurons contain NGF receptors and respond to NGF during their entire lifetime."

The findings attracted a great deal of interest because the cholinergic tracts that respond to NGF in the animals correspond to the major nerve tracts found to degenerate in the brains of Alzheimer's patients. Moreover, although there are other neuronal abnormalities, the loss of the cholinergic tracts correlates best with the patients' memory losses. This suggested that preserving those tracts might help the patients. The logic was straightforward, says William Mobley of the University of California, San Francisco: "NGF saves neurons; those neurons are dying in Alzheimer's brains; let's give NGF."

Early animal studies indicated that the logic may be correct. In one study, for example, Gage and his colleagues identified aged rats that had a lower than normal ability to learn to swim a water maze. NGF injections into the animals' brains both improved their learning behavior and also partly reversed the atrophy of the cholinergic neurons seen in the aged animals.

In fact, when the researchers first discovered NGF's activities in brain, they thought a deficiency of the protein might be the long-sought cause of Alzheimer's disease. They have since found no evidence to substantiate that hypothesis. "In retrospect, the

hypothesis that lack of NGF causes neuronal degeneration was naive," Hefti remarks.

But NGF might be a useful drug for treating Alzheimer's patients, even if it is not involved in any way in the etiology of the disease. "The application of NGF could still make the cholinergic neurons stronger and healthier and more resistant to disease," says Hefti. "People are not saying that it's a cure," Gage concurs, "or that it treats the cause, but it may treat one of the symptoms."

No one expects that the growth factor will be able to restore connections that are already lost, either. The principal goal is to stave off further deterioration. Since Alzheimer's usually develops late in life, simply buying a decade or two of time before the mental deterioration becomes severe may be all that is needed.

In any event, in the summer of 1988, the NIA convened a study group to consider whether there is a convincing rationale for using NGF to treat Alzheimer's disease. They concluded that the answer was yes—but set several conditions that would have to be fulfilled before the go-ahead could be given.

Those conditions are now beginning to be met. One of the easiest to fulfill, at least from the scientific point of view, was the development of an adequate supply of well-characterized human NGF for carrying out the clinical trials. Two companies, Syntex Corporation of Palo Alto and Genentech, Inc., of South San Francisco, have cloned the human NGF gene, which they can use to synthesize the protein. The recombinant proteins behave very much like natural NGF, Hefti and Gage say.

The NIA study group also wanted studies of NGF's activities in the brains of nonhuman primates. The early work had been done with rats, and there was always the possibility that NGF's brain effects might be unique to that species. But that is not the case, according to new data presented by Gage at a workshop on "New Strategies for the Treatment of Alzheimer's Disease," which was held on 9 and 10 January at the National Institutes of Health in Bethesda, Maryland. Gage, with his colleagues David Amaral, Ben U, and Mark Tuszynski, cut a cholinergic pathway in the brains of macaques, just as had been done previously in rats, and showed that the neurons degenerate—unless NGF is injected into the animals' brains. "Mouse NGF can protect these cholinergic neurons, too," Gage says. "This is the first demonstration that NGF works in a monkey."

The researchers now plan to assess NGF's action in aged primates. Donald Price of Johns Hopkins University School of Medicine has found that the brains of these animals can show the same kinds of abnormalities that occur in Alzheimer's brains. And the resemblance is not just limited to the physical changes. "We've characterized them [the aged animals]; some are very impaired on a variety of memory tasks," Price says. If NGF improves the memory performance of the animals, without causing unacceptable toxicity, it would certainly give impetus to the movement to test NGF in human Alzheimer's patients.

The NIA study group also called for more toxicity studies on NGF. The potential toxicity of NGF remains one of the thorniest issues to be resolved, especially since the protein would have to be administered over months or years for Alzheimer's therapy. As mentioned previously, some investigators have proposed that neuronal growth might actually be part of Alzheimer's pathology, raising the possibility that NGF administra-

Can Nerve Growth Be Detrimental?

The memory losses and other mental deterioration suffered by Alzheimer's patients are generally attributed to the extensive nerve cell degeneration that occurs in the patients' brains. But some investigators have evidence suggesting that increased neuronal growth may actually be contributing to the nerve cell losses. And that, cautions Larry Butcher of the University of California, Los Angeles, means that giving nerve growth factor to the patients might actually make their conditions worse.

He think that the environment in Alzheimer's brains already fosters excessive neuronal growth, which may detrimental. "You can't get stable [neuronal] networks formed if the growth is running amok," Butcher says. Inability to form stable connections between nerve cells might impair memory formation and might also lead to the neuronal degeneration. Many neurobiologists think that neurons cannot survive unless they form stable connections.

Carl Cotman and his colleagues at the University of California, Irvine, have also detected abnormal sprouting of cholinergic fibers in brains from Alzheimer's patients, possibly indicating an attempt of surviving nerve cells to form new connections to make up for neuronal losses.

But the sprouting may make matters worse by participating in the formation of the abnormal plaques found primarily in those brain regions where the heaviest neuronal losses occur. These plaques consist of deposits of a protein, called β-amyloid, surrounded by tangles of degenerating nerve endings called neurites. Although there is still a great deal of uncertainty about whether the plaques are the cause or the result of the neuronal damage, it is safe to say that nobody thinks that they are doing the brain any good.

In recent work, the Cotman group has found that β-amyloid can stimulate neuronal branching in culture. Such dendritic sprouting may also be taking place in the plaques themselves, thereby forming the neuritic tangles, according to Y. Ihara of the Tokyo Metropolitan Institute of Gerontology.

These observations have led Cotman to suggest that a vicious cycle is taking place in the developing plaques. β-amyloid, which is derived from a normal membrane protein made by nerve cells, may be deposited as a result of neuronal damage. It could then elicit neurite outgrowth from nearby neurons. And then this could lead to the death of more nerve cells by cutting them off from their connections and still more β-amyloid deposition, Cotman proposes.

And William Mobley of the University of California, San Francisco, gave NGF's supporters another cause for concern at the end of 1988 when he and his colleagues found that NGF itself stimulates the activity of the gene encoding the precursor protein that contains β-amyloid. "We were all very scared," says USC's Franz Hefti. "That could accelerate the disease process."

So far at least, no indication that that is happening has turned up in studies in which NGF has been injected into rat or primate brains. With the exception of one 5-month experiment conducted by Hefti, however, these have all been short, lasting no longer than 6 weeks or so.

No one thinks that the toxicology studies to date are sufficient to allay the concerns that have been raised about NGF's effects, let alone to satisfy the requirements that the U.S. Food and Drug Administration is likely to impose before human trials can be conducted. Says Fred Gage of the University of California, San Diego, "All of us feel that it's important to do toxicology studies to see if there are untoward effects of NGF." ■ **J.M.**

tion might speed up the patients' deterioration.

Should the toxicology studies pan out, another problem would have to be addressed—how to administer the NGF. It is a protein and proteins are not supposed to cross the blood-brain barrier. The current thinking is that NGF would be delivered through a fine tube inserted directly into a patient's brain and connected to a small pump that could infuse a solution containing the protein.

But the invasiveness of this procedure might well make it difficult to perform a standard controlled study with some patients getting NGF and others an inert material. Hefti points out that it would probably be unethical to outfit a patient with a pump and then not give him or her an active drug. Some clinical trials have gotten around this type of problem by letting patients serve as their own controls, alternately giving them the active agent and placebo control. This might be done with NGF. The animal studies have indicated, however, that the growth factor's effects may persist for days or weeks after administration is stopped, and this persistence could be another complicating factor if the protein were to be alternated with a placebo control.

The researchers are also looking for alternatives to direct brain injection. Mobley's group, for example, is beginning to identify the segments of the NGF protein that are required for its activity. Eventually, this kind of information might be used to design nonprotein drugs that could cross the blood-brain barrier on their own. Another approach, Khachaturian says, would be to encapsulate the protein or active peptides in a membrane that could also penetrate the blood-brain barrier.

And then there is the prospect of genetic engineering. In one set of experiments, for example, Gage and his colleagues introduced an active NGF gene into rat fibroblasts, a type of cell that would not normally make NGF. They then implanted the cells into the brains of rats that contained their standard lesion in the cholinergic nerve tract running between the basal forebrain and hippocampus. The NGF-secreting cells prevented the cholinergic nerves from degenerating, just as the NGF injections did.

But for now, researchers are concentrating on direct NGF infusion, not genetic engineering. Next month, Khachaturian will convene another study group at NIA to assess the situation again. Despite the great need for an Alzheimer's treatment, no one wants to make a mistake now. "It's more than ordinary drug development," Hefti says. "We are dealing with an entire new concept." ■ JEAN MARX

Shooting at a New HIV Target

The first reports are trickling in from a new front in the war against AIDS, and the news is promising. Two teams, one at Smith Kline & French Laboratories in King of Prussia, Pennsylvania,* and another at the Upjohn Company in Kalamazoo, Michigan (see page 454, this issue), have identified compounds that inactivate a crucial enzyme that the AIDS virus, HIV, needs to reproduce itself. Although the work has not yet gone beyond the test tube, these companies, as well as numerous others around the world, are now working feverishly to find even more potent compounds that may ultimately be used therapeutically to limit HIV infection in humans.

The targeted enzyme—called HIV protease—breaks two large viral polyproteins into smaller units. Some of these are assembled together to form the virus's core; others form molecules that permit HIV's genetic material to insinuate itself into a healthy cell's nucleus.

Efforts to design compounds to attack HIV protease rely on knowledge gained from the explosion of research on the basic biology of the virus. But they have also benefited from a bit of luck—the fact that pharmaceutical companies have been struggling for a decade to find a way to block renin, an enzyme with some similar properties that plays a critical role in raising blood pressure.

Although the precise three-dimensional structure of HIV protease has only recently been accurately determined (*Science*, 11 August 1989, p. 616), it has been known for some time that, like renin, it is an aspartic protease—so called because two aspartic acid residues flank the active site of the enzyme. As Alexander Wlodawer, a crystallographer at the National Cancer Institute who has been studying the three-dimensional structure of HIV protease, puts it, "If it weren't for [the work on] renin inhibitors, we wouldn't be anywhere right now with HIV protease inhibitors."

"We had molecules that were potent inhibitors of renin," says W. Gary Tarpley who directed the Upjohn effort. "We systematically modified various regions of some of our renin inhibitors so that we could optimize the protease activity."

It was clear from assays that the new compounds had a direct effect on the protease, but would they enter cells and seek out the enzyme? For both groups, the answer was yes. Both groups have also shown that in the presence of the antiprotease compounds, the virus can form new viral particles, but the newly created viruses are incapable of infecting other cells.

Promising though these preliminary tests are, several hurdles must still be overcome to develop a candidate compound for human therapy. Tarpley says Upjohn's compound can be washed out of infected cells and its effect mitigated. He says compounds with longer half-lives will have to be found.

Then there is the question of specificity. Any effective drug will have to work only on HIV protease and not other aspartic proteases. According to John Kay, a protein chemist at the University of Cardiff, five human aspartic proteases (or proteinases, as he insists they are more properly called) have been identified: renin, two digestive enzymes called pepsin and gastricsin, and two proteolytic enzymes called cathepsin E and cathepsin D. Part of the difficulty pharmaceutical companies encountered in developing oral anti-renin compounds is that they also attacked pepsin and gastricsin, which are found in the stomach. More problematic for AIDS therapy may be the fact that cathepsin E plays a role in normal immune functioning.

"If you start giving [AIDS patients] a drug which interferes in its side effects with another enzyme that's an aspartic proteinase that's involved in immune surveillance and antigen processing, then that's really bad news," says Kay.

A British company may, however, have cracked the specificity problem. Roche Products, the British subsidiary of Hoffmann–La Roche, has discovered a group of compounds that they say are much more selective for the HIV protease than for any of the human aspartic proteases.

The Upjohn and Smith Kline teams have taken the first step in showing that targeting HIV protease may be an effective AIDS therapy. "Now it's a question of taking something which works in tissue culture and making it into a drug," says Wlodawer. "That's a big step, but without these results, there would be no step to take." ■ JOSEPH PALCA

* T. D. Meek *et.al.*, *Nature* **343**, 90 (1990).